POSTER COMMUNICATIONS

Sulphinpyrazone in rats releases prostaglandin 'reciprocal coupling factor': possible mode of anti-thrombotic action?

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The platelet-sparing and anti-thrombotic effects of sulphinpyrazone have prompted extensive trials for the prevention of sudden death after myocardial infarction. However, the mechanisms whereby sulphinpyrazone inhibits platelet aggregation *in vivo* are not yet fully elucidated. It does not seem to be a powerful aspirin-like inhibitor of platelet prostaglandin (PG) and thomboxane synthesis (Margulies & White, 1979). We show here that in rats small doses of sulphinpyrazone cause the release of prostaglandin 'reciprocal coupling factor' (RCF).

RCF is the provisional name we have given to the component(s) in plasma which *in vitro* test systems inhibits synthesis of classical PGs by microsomal preparations and enhances PG degradation in 100,000 *g* cytosolic supernatants (Moore & Hoult, 1980). Levels of RCF are altered in several pathophysiological states in which PGs are involved, and we have proposed that RCF may be an important regulator of the activity of the PG system.

In these experiments, RCF was measured in standardized tests of the ability of rat plasma to inhibit PG synthesis by bovine or sheep seminal vesicle microsomes and to stimulate PG metabolism by freshly prepared 100,000 *g* supernatants of rat caecum homogenates, using methods described in Hoult & Moore, 1980. Male Sprague-Dawley rats were anaesthetized with urethane (650 mg/kg i.p. and s.c.) and cannulated at the carotid artery for making injections and removing blood samples.

In four separate series of experiments, each with three rats, we found that i.a. injection of sulphinpyrazone (50 μg/kg), but not saline, produced a large increase in plasma RCF activity according to both the synthesis-inhibition and metabolism-stimulation assays. Blood was sampled at 60 min only, and the time course of release is not known. Plasmas were tested at 0.5, 5.0 and 10.0% v/v. For example at 5% control plasma inhibited PG synthesis by 51.0 ± 2.8% ($n = 76$) and this was increased to 67.4 ± 3.3% ($n = 38$) by plasma from sulphinpyrazone-treated animals ($P < 0.001$). Breakdown of $PGF_{2\alpha}$ by rat colon was stimulated 42.1 ± 5.8% ($n = 39$) by control plasma and this was increased by 'sulphinpyrazone' plasma to 67.3 ± 5.8% ($n = 22$, $P < 0.01$).

Preliminary experiments showed that 'sulphinpyrazone' plasma enhanced prostacyclin production by washed rat aortic rings to a larger extent than control plasma. Prostacyclin was assayed by inhibition of ADP-induced human platelet aggregation.

These results show that sulphinpyrazone at therapeutically relevant doses releases a plasma factor which has far-reaching effects on the PG system. A reduction in PG synthesis and enhancement of degradation plus stimulation of prostacyclin synthesis may contribute directly to the anti-thrombotic effects of this drug.

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Arachidonate metabolites play only a minor role in collagen-induced aggregation in mouse citrated platelet-rich plasma

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A study of mouse platelet aggregation and its inhibition *in vitro* showed that aspirin and flurbiprofen were ten fold and one hundred fold respectively less potent as inhibitors of collagen-induced aggregation in mouse citrated platelet-rich plasma (PRP) than human citrated PRP (Nunn, 1980). These two drugs are thought to inhibit aggregation of human platelets by inhibiting cyclo-oxygenase so preventing the conversion of endogenous arachidonate to the potent aggregating agents, prostaglandins G_2 and H_2 and thromboxane A_2 (Hamberg, Svensson, Wakabayashi & Samuelsson, 1974). The results in mouse PRP therefore suggested that arachidonate metabolites play little part in the response of mouse platelets to collagen. The present experiments were carried out in order to investigate this further.

Male mice (35–45 g) were placed in a chamber of CO_2 until respiration ceased. Blood (0.9 ml) was drawn from the inferior vena cava and mixed with trisodium citrate (0.1 ml, 129 mM). Citrated blood from four or five mice was pooled and centrifuged at 450 *g* for 6 min at 22°. After transfer of the PRP, the infranatant was centrifuged at 2600 *g* for 20 min to prepare platelet-poor plasma (PPP). Platelet concentration was determined using a Thrombocounter C (Coulter Electronics Ltd.) and adjusted to 10^6 platelets/μl PRP with PPP. Arachidonic acid (Grade 1, Sigma) was dissolved in Na_2CO_3 (50 mM) and stored at −20°. Collagen (Hormon-Chemie, Munich) or arachidonate (0.4–5 μl) was added to 0.1 ml stirred PRP at 37° in an aggregometer (Albert Browne Ltd., Leicester) after 3 min incubation with NaCl (0.1 vol, 154 mM) as control or aspirin freshly dissolved in NaCl (154 mM). Aggregation responses were quantified as changes in light transmission (Born, 1962). Concentrations of collagen and arachidonate producing a 50% maximal response (EC_{50}) were estimated from $\log_{10}$ concentration-response curves. Dose-ratios were calculated from the ratio of EC_{50}'s in the presence and absence of aspirin.

It was found that arachidonate caused aggregation in mouse PRP, its potency being similar to that in human PRP in this laboratory (EC_{50} = 274 ± 67 μM, mean ± s.e. mean, n = 8). Aspirin, at concentrations (200–400 μM) sufficient totally to block the response to 1 mM arachidonate, had only a minor inhibitory effect on responsiveness to collagen in mouse PRP (dose-ratio = 1.35 ± 0.05, mean ± s.e. mean, n = 4).

These results show that mouse platelets can aggregate in response to arachidonate and that aspirin can inhibit cyclo-oxygenase in mouse PRP. The weakness of the inhibitory effect of aspirin on collagen-induced aggregation in mouse PRP would therefore appear to be due to poor activation by collagen of the arachidonate pathway. The question arises of what other factors could be responsible for mediating collagen-induced aggregation in mouse PRP. It cannot be attributed to released diphosphate (ADP), for, unlike responses to collagen in the presence or absence of aspirin, aggregation responses to ADP in mouse PRP undergo very rapid disaggregation (Nunn, 1980). Hence, other mediators must be involved.

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Prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) mediated contraction and [^{45}Ca]-influx into rat mesenteric arteries: inhibition by flunarizine a calcium entry blocker

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The calcium entry blocker flunarizine (Godfraind, 1979) has been utilized to study the source of the calcium mobilized by $PFG_{2\alpha}$ to produce contraction in a rat mesenteric artery spiral strip preparation and to investigate membrane calcium channels. The physiological solution used for the contractile experiments contained (mM) NaCl 112, KCl 5, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1, $CaCl_2$ 1.25 and glucose 11.5 and for radioactive calcium uptake measurements the composition (mM) was NaCl 122, KCl 5.9, $NaHCO_3$ 15, $MgCl_2$ 1.25, $CaCl_2$ 1.25 and glucose 11. Both solutions were equilibrated with a mixture of 95% O_2 and 5% CO_2 at 37°C. Reproducible cumulative concentration effect curves in rat mesenteric artery strips were produced by $PGF_{2\alpha}$ when separated by 90 minutes.

$PGF_{2\alpha}$ (33.6 μM) stimulates influx of [^{45}Ca] into the rat mesenteric artery as measured by the method of Godfraind (1976). The threshold concentration of $PGF_{2\alpha}$ producing contraction was 0.2 μM and the maximum effect was obtained with 33.6 μM. Flunarizine (10^{-8}–10^{-6} M) produced dose related depression of $PGF_{2\alpha}$ responses (concentration producing 50% depression of maximal response (IC_{50}) 1.7×10^{-7} M), with no apparent shift to the right of the concentration effect curves. Flunarizine 10^{-5} M inhibited responses completely. $PGF_{2\alpha}$ stimulated influx of [^{45}Ca] into the rat mesenteric artery was reduced in a dose dependent manner by flunarizine 10^{-8}–10^{-6} M (IC_{50} 1.4×10^{-7} M) which itself had no significant effect on [^{45}Ca]-uptake.

The flunarizine IC_{50} values for inhibition of contraction and [^{45}Ca]-influx are close, suggesting that blockade of calcium entry is mainly responsible for the inhibitory effect on contraction. Since flunarizine 10^{-5} M completely inhibited the $PGF_{2\alpha}$ induced contraction it is likely that it relies entirely on the entry of extracellular calcium.

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A possible role for lipoxygenase products in airway hyper-reactivity *in vivo*

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The airways of asthmatics are often hyper-reactive to a variety of stimuli, including spasmogens such as histamine, acetylcholine and $PGF_{2\alpha}$ (Mathe, Hedgvist, Holmgren & Svanberg, 1973). The precise mechanism of this non-specific hyper-reactivity is still undefined, but a possibly related phenomenon has been observed in guinea-pig isolated trachea treated with cyclo-oxygenase inhibitors (Orehek, Douglas & Bouhuys, 1975). Orehek *et al.* (1975) attributed such hyper-reactivity to decreased production of a relaxant prostaglandin, such as PGE_2. More recently, Adcock & Garland (1980) demonstrated that indomethacin-induced hyper-reactivity to histamine in isolated guinea-pig trachea could be prevented but not mimicked by dual inhibitors of cyclo-oxygenase and lipoxygenase and suggested that increased synthesis of a lipoxygenase product may be responsible for the hyper-reactivity.

In the present studies, hyper-reactivity was induced in conscious guinea-pigs by aerosol administration of either cyclo-oxygenase inhibitors or 13-HPLA (13-hydroperoxy-linoleic acid), a stable lipid peroxide used in an attempt to mimic hydroperoxide products of lipoxygenase. Male Dunkin-Hartley guinea-pigs (300–400 g) housed in individual perspex boxes were exposed for 10 min to Wright's nebuliser aerosols of histamine, at a concentration (100 μg/ml in phosphate buffered saline) that produced threshold responses in

control animals. Symptoms of respiratory distress during histamine exposure were assessed on an eight-point scale by observing and timing abdominal panting, cyanosis, spasms and collapse. In each experiment, treatments were allocated to groups of six animals using a randomized block design and symptoms scored 'blind' by an independent observer. Drug aerosols were administered by the same system for a period of 1 s/g body weight (i.e. 5–6½ minutes for a 300–400 g pig) starting at a fixed time before histamine.

A marked hyper-reactivity was demonstrated when cyclo-oxygenase inhibitors, indomethacin (3–30 mg/ml), meclofenamic acid (3–30 mg/ml) and aspirin (100–200 mg/ml) were administered immediately before histamine, the potency ratios obtained being compatible with cyclo-oxygenase inhibitor activities (Flower, 1974). Pretreatment with a dual cyclo-oxygenase/lipoxygenase inhibitor, BW755C (Higgs, Flower & Vane, 1979) (10–30 mg/kg i.p. 60 min before histamine) left control responses unchanged but abolished the induced hyper-reactivity. 13-HPLA (1–3 mg/ml), similarly administered immediately prior to histamine, produced a very marked increase in response which was unchanged either by BW755C or CLI, a related dual cyclo-oxygenase/lipoxygenase inhibitor (Adcock, Garland, Moncada & Salmon, 1978), confirming that its action was independent of endogenous arachidonic acid metabolism.

This apparently direct effect of lipid hydroperoxide and the evidence presented that a similar hyper-reactivity can be induced by diversion of substrate arachidonic acid from cyclo-oxygenase to lipoxygenase metabolism, supports the hypothesis that lipoxygenase products may be involved in airway hyper-reactivity *in vivo*.

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The effects of arachidonate lipoxygenase products on leukocyte migration in rabbit skin

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The arachidonate lipoxygenase product 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) is chemotactic for polymorphonuclear leukocytes (PMNs) (Turner, Tainer & Lynn, 1975) and it has been suggested that lipoxygenase products are mediators of leukocyte accumulation in the inflammatory response. 5-HETE synthesized by PMNs is also chemotactic (Goetzl & Sun, 1979) but the di-hydroxy acid 5,12-diHETE is the most potent chemokinetic and chemotactic lipoxygenase product so far tested (Ford-Hutchinson, Bray, Doig, Shipley & Smith, 1980; Palmer, Stepney, Higgs & Eakins, 1980; Smith, Ford-Hutchinson & Bray, 1980). In this study we have compared the effects of lipoxygenase products, arachidonic acid and stable prostaglandins from the E series on leukocyte accumulation in rabbit skin.

Hydroperoxy acids (HPETEs) were synthesized by the oxygenation of arachidonic acid (Porter, Wolf, Yarbro & Weenan, 1979) and the corresponding hydroxy acids (HETEs) were prepared by reduction with triphenyl phosphine. 5,12-diHETE was synthesized by incubating 2×10^9 glycogen-stimulated rabbit peritoneal leukocytes with arachidonic acid (1 mg) and

ionophore A23187 (50 μg) (Borgeat & Samuelsson, 1979). Products were separated by high performance liquid chromatography and identified by gas-liquid chromatography and mass spectrometry.

Leukocyte infiltration was estimated after intradermal injection of arachidonate metabolites (in 100 μl 50 mM Tris buffer, pH 7.5) into the shaved backs of New Zealand White rabbits (2–3 kg). The animals were killed after 1–4 h, the skin removed and injection sites separated using a $\frac{5}{8}$" steel punch. Skin samples were processed histologically and sections (4–5 μm) cut at four different points of each injection site. The sections were stained with haematoxylin and eosin before microscopic examination at low power (×160) to locate the injection site. Leukocyte numbers were counted in five high power (×1000) fields (hpf) arranged vertically through the dermis. Each treatment was given at 2–6 injection sites in at least three animals.

5,12-diHETE (25–100 ng) caused a dose-dependent increase in the numbers of leukocytes in the dermis which was significantly greater than the response caused by injection of Tris buffer alone. Skin receiving Tris buffer contained 136 ± 11 leukocytes per 5 hpf (mean ± s.e. mean, $n = 51$) at 4 h, whereas skin injected with 100 ng 5,12-diHETE contained 454 ± 110 ($n = 9$) cells. The leukocytes counted were predominantly PMNs. An enhancement of infiltration was also seen with arachidonic acid (10 μg), 12-HPETE (1 μg), 12-HETE (1 μg) or PGE_1 (1 μg) but each of these were at least 20 times less potent than 5,12-diHETE. PGE_2 (0.1–1 μg) 5-HPETE (0.1–1 μg) or 5-HETE (0.1–1 μg) did not increase cell numbers.

These results indicate that 5,12-diHETE could be involved in the control of leukocyte migration in the inflammatory response. This activity coupled to its ability to increase vascular permeability and enhance bradykinin-induced plasma exudation (Eakins, Higgs, Moncada, Salmon & Spayne, 1980) suggests that 5,12-diHETE may be an important inflammatory mediator.

The mono-hydroxy acids were provided by R.J. Stepney.

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Chemotactic activity of arachidonic acid lipoxygenase products in the rabbit eye

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Polymorphonuclear leukocytes (PMN) metabolize arachidonic acid to 5,12-diHETE (Leukotriene B) (Borgeat & Samuelsson, 1979), which has been shown *in vitro* to be a potent chemokinetic (Ford-Hutchinson, Bray, Doig, Shipley & Smith, 1980) and chemotactic compound (Palmer, Stepney, Higgs & Eakins, 1980). In the present experiments we have compared the chemotactic activity of a variety of products of the lipoxygenase pathway and the synthetic chemotactic peptide F-met-leu-phe following their injection into the anterior chamber of the rabbit eye.

New Zealand white rabbits weighing between 2 and $2\frac{1}{2}$ kg were anaesthetized with urethane (1.5 g/kg i.v.). Mean arterial blood pressure was monitored from a femoral artery. The cornea of each eye was transfixed with a needle (23 g) having a hole on each side of a central occlusion as described by Nagasubramanian

Table 1 Leucocyte migration into the aqueous humour* following intracameral injection of 5,12-diHETE and F-met-leu-phe

	Dose ng	Leucocytes/mm³ in Aqueous Humour: Control Eye	Leucocytes/mm³ in Aqueous Humour: Test Eye	Number of Animals	Statistical significance t-test (P)
5,12-diHETE	25	66 ± 20	424 ± 163	4	n.s.
	200	65 ± 18	1572 ± 276	5	<0.01
	400	51 ± 29	1480 ± 149	4	<0.01
F-met-Leu-phe	100	39 ± 3	389 ± 129	4	n.s.
	250	282 ± 241	1150 ± 200	4	<0.01
	500	50 ± 25	1027 ± 105	5	<0.01

Values are expressed as means ± s.e. mean.
* Samples of aqueous humour were withdrawn 4 h after the intracameral injections.

(1974). Each end of the needle was connected by polyethylene tubing to a pressure transducer and reservoir *via* a closed circuit perfusion system to permit continuous recording of IOP and to make injections. Known amounts of compounds under investigation were dissolved in 25 µl saline and loaded into one half of the perfusion system. All compounds were injected by manipulating the perfusion system (saline in the contralateral eye) following 10 min recording of IOP, the pump was then turned off and IOP recorded for the duration of the experiment. After 4 h the animal was killed with an overdose of sodium pentobarbitone and the aqueous humour removed from each eye and total leukocyte numbers estimated on a double-blind basis.

The introduction of either 5,12-diHETE or F-met-leu-phe resulted in a dose-dependent increase in total white cell counts in the aqueous humour. The results are seen in Table 1. Injection of 500 ng in groups of four animals of 5-HPETE, 5-HETE, 12-HETE, prostaglandin E_2 (PGE_2) and PGE_1 were not chemotactic, but PGE_2 and PGE_1 resulted in an increase in IOP (up to 40–50 mmHg) following the injection. Doses of up to 25 µg arachidonic acid raised the IOP but did not increase the leukocyte count.

It is concluded that Leukotriene B is a chemotactic agent *in vivo* with a potency comparable to that of F-met-leu-phe. The other lipoxygenase products, together with PGE_2, PGE_1 and arachidonic acid, were not chemotactic in this model. PGE_2, PGE_1 and arachidonic acid were the only compounds to increase the IOP, an effect which is thought to result from an increase in the permeability of the blood/aqueous barrier (Beitch & Eakins, 1969; Bhattacherjee, Kulkarni & Eakins, 1980). In addition the rabbit eye would appear to be a useful model for chemotactic studies *in vivo*.

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The vasoactive effects of some arachidonate lipoxygenase and cyclo-oxygenase products on the isolated perfused stomach of rabbit and rat

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The gastric vascular actions *in vitro* of various arachidonic acid metabolites formed by the cyclo-oxygenase enzyme system have previously been investigated using the isolated vascular-perfused stomach of rabbit (Salvati & Whittle, 1980). We have now compared the vasoactive effects of the cyclo-oxygenase products, prostacyclin (PGI_2) and prostaglandin E_2 (PGE_2) with some of the hydroperoxy and hydroxy lipoxygenase products of arachidonic acid in the isolated perfused stomach of both the rabbit and the rat.

The gastric circulation of the rabbit stomach was prepared as described previously (Salvati & Whittle, 1980). All branches of the splenic artery and vein (excluding the left gastro-epiploic vessels) and the branches of the coeliac artery supplying the liver, duodenum and pancreas were ligated. Krebs solution (37°C; gassed with O_2 95%, CO_2 5%) containing in the current study 0.02% dextran (molecular weight 5×10^6) was perfused (12.5 ml/min) through the vasculature via a catheter inserted into the coeliac artery, and the perfusate collected from a cannula in the hepatic portal vein. The vasculature of the rat stomach was prepared and perfused *in vitro* in a comparable fashion but at 5 ml/min. Vascular resistance was measured as changes in perfusion pressure via a transducer connected to the coeliac catheter and drugs were injected into the catheter close to the stomach. The hydroperoxy products of arachidonic acid were prepared chemically by autoxidation (Porter, Wolf, Yarbro, Weenen, 1979), subsequently reduced to the hydroxy acids with triphenyl phosphine and separated by high performance liquid chromatography and identified by gas chromatography–mass spectrometry (Palmer, Stepney, Higgs & Eakins, 1980).

To demonstrate clearly the actions of the vasoactive substances, the basal perfusion pressure (30–50 mmHg for both rat and rabbit stomach) was increased (by 40–60 mmHg) by infusion of noradrenaline (1–5 μg/min). Bolus injections of prostacyclin (2.5–50 ng) or PGE_2 (25–100 ng) caused a dose-dependent fall in PP, in both preparations. In the rabbit stomach prostacyclin (5 ng) reduced PP by 21 ± 2 mmHg (mean ± s.e. mean, $n = 3$) and PGE_2 (100 ng) by 20 ± 3 mmHg. Likewise, in the rat stomach, prostacyclin (2.5 ng) caused a fall of 22 ± 6 mmHg and PGE_2 (100 ng) a fall of 20 ± 5 mmHg ($n = 4$).

Bolus injections of the hydroperoxy acids, (0.5–4 μg) also caused dose-dependent falls in the noradrenaline-elevated vascular tone in both preparations. In six experiments in the rabbit stomach, the fall in PP was 17 ± 2 mmHg with 12-HPETE (2 μg), 11 ± 0.5 mmHg was 5-HPETE (2 μg) and 15 ± 2 mmHg with 15-HPETE (4 μg). In three experiments in the rat stomach the fall in PP was 20 ± 2 mmHg with 12-HPETE (2μg), 10 ± 3 mmHg with 5-HPETE (2 μg) and 12 ± 1 mmHg with 15-HPETE (4 μg). In both preparations, the hydroxy acids 12-HETE and 5-HETE (2 μg) had little activity on vascular resistance.

The present results indicate that as in the rabbit stomach, prostacyclin and PGE_2 are both vasodilators in the rat stomach *in vitro*, with prostacyclin being at least 40 times the more potent. Previously, PGE_2 has been reported to have vasoconstrictor actions in the rat isolated perfused kidney (Malik & McGiff, 1975) and mesentery (Malik, Ryan & McGiff, 1976; Manku, Matabaji & Horrobin, 1977). Although the lipoxygenase mono-hydroxy acids so far investigated had little vascular activity, the hydroperoxy acids had vasodilator actions, but were less active than the cyclo-oxygenase products prostacyclin or PGE_2. Nevertheless, if such lipoxygenase products are generated by the gastric mucosa, they could play some role in modulating local gastric blood flow, especially under conditions of reduced cyclo-oxygenase activity. Whether actions on the metabolism of arachidonic acid by the lipoxygenase enzymes as well as by the cyclo-oxygenase enzymes can be implicated in the gastro-intestinal toxicity of high doses of anti-inflammatory drugs can only be speculated on.

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Do prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2) affect disruption of the gastric mucosal barrier induced by bile salt?

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In the rat PGE_2 and its stable methylated derivative, 16,16-dimethyl PGE_2, protect the gastric mucosa against extreme forms of experimental injury (Robert, Nezamis, Lancaster & Hanchar, 1979). The mechanism of this protection is unknown, but prostanoids may act by modifying the permeability of the mucosa to the passive flux of ions (Bommelaer & Guth, 1979). We have investigated the effect of intravenously infused PGE_2 and PGI_2 on changes in gastric mucosal integrity induced by topical bile salt as indicated by measurement of transepithelial potential difference (p.d.), H^+ loss from and Na^+ and K^+ gain into the gastric lumen.

In urethane-anaesthetized rats (170–210 g) the gastric lumen was filled with 4 ml of an acidic solution which was replaced at 30 min intervals. Each experiment consisted of two control periods, followed by a test period and then two control (recovery) periods. During control periods the acidic solution consisted of HCl (100 mM), mannitol (80 mM), NaCl (10 mM) and polyethylene glycol-4000 (5 g/l containing 1 μCi/l [^{14}C]-PEG-4000) as a volume marker; during test periods NaCl was replaced with sodium taurocholate (NaTC).

In six control experiments where no NaTC was added the mean values for the measured parameters for each of five 30 min periods were within the following ranges: p.d. (–mV) 56.3–57.3; H^+ loss (μeq/30 min) 36.5–39.9; Na^+ gain (μeq/30 min) 11.4–15.6; K^+ gain (μeq/30 min) 1.1–3.0. Instillation of 5 mM NaTC into the stomach had little effect on p.d. and net ion flux, whereas 10 mM NaTC produced large changes: p.d. (–mV) changed from 57.3 ± 1.4 to 35.3 ± 1.3; H^+ loss (μeq/30 min) increased from 37.2 ± 6.7 to 102.1 ± 9.3; the influx of Na^+ and K^+ ions (μeq/30 min) increased from 11.8 ± 2.4 to 61.5 ± 7.6 and from 1.4 ± 0.5 to 4.1 ± 0.4 respectively (mean ± s.e. mean, $n = 6$ throughout).

PGE_2 and PGI_2 inhibited histamine-stimulated gastric acid secretion in the anaesthetized rat, and the ED_{50} values were 3.9 and 0.8 nmol kg^{-1} min^{-1} i.v. respectively. PGE_2 at doses of 0.3, 3 and 30 nmol kg^{-1} min^{-1} i.v. had no overall effect on the changes in p.d. and ion flux induced by NaTC (10 mM) as would be expected with a protective agent. A similar result was obtained using PGI_2 at doses of 0.03 and 0.3 nmol kg^{-1} min^{-1} i.v., although a higher dose of PGI_2 (3 nmol kg^{-1} min^{-1} i.v.) prevented the recovery of the gastric mucosa from the damaging effects of 10 mM NaTC.

The present results provide no evidence that PGE_2 and PGI_2 ameliorate the changes in p.d. and net ion flux induced in rat gastric mucosa by NaTC. Puurunen (1980) has also reported that PGE_2 does not affect the increased rate of acid loss from the gastric lumen caused by topical application of ethanol to rat gastric mucosa.

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Further evidence that AH 19437 is a specific thromboxane receptor blocking drug

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We have previously reported that on smooth muscle, AH 19437 selectively blocks that prostanoid receptor which is characterized by a high sensitivity to the PGH_2 analogue U-46619 (Coleman, Collington, Geisow, Hornby, Humphrey, Kennedy, Levy, Lumley, McCabe & Wallis, 1980). Furthermore we believe this receptor to be a thromboxane receptor (Coleman, Humphrey, Kennedy, Levy & Lumley, 1980a), and have provided preliminary evidence that AH 19437 blocks smooth muscle responses to TxA_2 (Coleman *et al.*, 1980). We now report a more detailed comparison of the effects of AH 19437 on responses of some smooth muscle preparations to TxA_2, U-46619 and PGH_2.

Rat aorta, dog saphenous vein, guinea-pig ileum, chick ileum and dog iris sphincter muscle were suspended for cascade superfusion at a rate of 5 ml/min with modified Kreb's solution containing antagonists (Coleman, Humphrey, Kennedy, Levy & Lumley, 1980b). PGH_2 and TxA_2 were generated enzymatically as described previously (Coleman *et al.*, 1980b).

TxA_2 (15–1500 ng), U-46619 (10–1000 ng) and PGH_2 (15–1500 ng) potently contracted rat aorta, dog saphenous vein and guinea-pig lung as previously described (Coleman *et al.*, 1980b). AH 19437 (2.4×10^{-5} mol/l) shifted dose-effect curves for all three agonists to the right by greater than 10-fold without affecting dose-effect curves to either acetylcholine or 5-hydroxytryptamine. Although precise quantification was not possible, dose-effect curves for all three prostanoids were shifted to approximately the same extent on each preparation. Although not proof, these results are consistent with the proposition that TxA_2, U-46619 and PGH_2 all act at the same receptor on these preparations.

PGH_2 (15–1500 ng) also potently contracted guinea-pig ileum, chick ileum and dog iris sphincter muscle, but these responses were unaffected by AH 19437, suggesting that on these preparations PGH_2, whether acting directly or by conversion to other prostanoids, does not act at the same receptor as in rat aorta, dog saphenous vein and guinea-pig lung. On chick ileum and dog iris sphincter muscle, TxA_2 and U-46619 were also unaffected by AH 19437. Surprisingly, responses of guinea-pig ileum to U-46619, but not TxA_2 were blocked by AH 19437. One possible explanation is that guinea-pig ileum contains only a small population of the U-46619/TxA_2-sensitive receptor, but that the action of TxA_2 on this preparation is complicated by contamination with small amounts of PGH_2.

In conclusion, the results of this study support our previous suggestions that the U-46619-sensitive receptor is a thromboxane-receptor and that AH 19437 is a specific thromboxane-receptor blocking drug.

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Leukotriene B_4: a mediator of vascular permeability

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Leukotriene B_4 (LTB_4; 5,12 dihydroxy eicosatetraenoic acid) is a mixture of isomers derived from arachidonic acid via an unstable intermediate leukotriene A_4 (Borgeat & Samuelsson, 1979). One of the isomers (III) is formed enzymatically and has been shown to have potent chemotactic, chemokinetic and aggregatory activity for leucocytes *in vitro* and to induce leucocyte migration *in vivo* (Ford-Hutchinson, Bray, Doig, Shipley & Smith, 1980; Smith, Ford-Hutchinson & Bray, 1980). In the present study we have examined the effects of LTB_4 on vascular permeability in rabbit, guinea-pig and rat skin and on blood flow in rabbit skin.

The isomer III of LTB_4 was prepared from rat polymorphonuclear leucocytes stimulated with ionophore A23187 as described previously (Ford-Hutchinson *et al.*, 1980). Changes in vascular permeability were monitored by measuring the extravasation of [^{125}I]-human serum albumin 30 min after the intradermal injection of LTB_4, in either the presence or the absence of 10 ng of PGE_2 (Williams, 1979). Changes in blood flow in rabbit skin were measured using the [^{133}Xe] clearance technique (Williams, 1979).

Doses of LTB_4 (from 1 to 100 ng per skin site), caused no change in blood flow in rabbit skin. In contrast, PGE_2 produced dose related increases in blood flow over the range 0.1 to 10 ng per skin site. Intradermal injections of either PGE_2 (10 ng) or LTB_4 (1, 10 and 100 ng) caused no significant increase in plasma exudation in the rabbit, guinea-pig or rat apart from a dose of 100 ng of LTB_4 which produced a small, but significant, increase in exudation in the rabbit skin (29 ± 8 µl of plasma exudate; Tyrode buffer alone 12 ± 2 µl). In the rabbit, when LTB_4 (1, 10 and 100 ng) and PGE_2 (10 ng) were administered together, significant plasma exudations were observed (20 ± 5 (1 ng), 78 ± 13 (10 ng) and 166 ± 13 (100 ng) µl of plasma exudate). Similar results were observed in the guinea-pig, where doses of 10 and 100 ng of LTB_4, in the presence of PGE_2 (10 ng), produced significant increases in plasma exudation and in the rat where only the highest dose of LTB_4 was active. These results demonstrate that LTB_4, in addition to its effects on leucocyte migration, causes increases in vascular permeability in the presence of a vasodilator such as PGE_2.

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Leukotriene B_4 and leucocyte movement

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Arachidonic acid is metabolized via a lipoxygenase pathway to leukotriene A_4 (Borgeat & Samuelsson, 1979a) which is further metabolized to a mixture of isomers of leukotriene B_4 (LTB_4; 5,12 dihydroxy eicosatetraenoic acid). Only one of these isomers (III) is enzymatically derived (Borgeat & Samuelsson, 1979b). Polymorphonuclear leucocytes (PMNs), exposed to ionophore A23187, release aggregating and chemokinetic activities for fresh PMN suspensions and these activities have been shown to be due to the release of LTB_4 (Ford-Hutchinson, Bray, Doig, Shipley & Smith, 1980). LTB_4 has also been shown to be produced by human eosinophils (Goetzl, Weller & Sun, 1980) and rat macrophages (Doig, Ford-Hutchinson & Bray, 1980).

LTB_4 (isomer III) and 5-HETE (5-hydroxy-eicosatetraenoic acid) were prepared from rat PMNS stimulated with ionophore A23187 (Ford-Hutchinson *et al.*, 1980). The chemokinesis of human PMNs, guinea-pig eosinophils, human monocytes and rat macrophages elicited with thioglycollate were measurd using an agarose microdroplet assay (Smith & Walker, 1980) using incubation times of $1\frac{1}{2}$, 2, 6 and 20 h respectively. The ED_{50} values for LTB_4 for these cell types were 1.0, 6.8, 11.3 and 4.2×10^{-10} M respectively. Table 1 shows the comparison of the effects of LTB_4 with those of the complement derived peptide C5a, F-met-leu-phe and 5-HETE on human PMN chemokinesis. The results show that LTB_4 has comparable activity on a molar basis to C5a and F-met-leu-phe and is 100 times more potent than 5-HETE. Other studies have shown that LTB_4 is also chemotactic for PMNs *in vitro* and when injected into the guinea-pig peritoneum *in vivo* stimulates the movement of PMNs

Table 1 Effects of LTB_4, C5a, F-met-leu-phe and 5-HETE on the chemokinesis of human PMNs

	ED_{50}
Leukotriene B_4 (Isomer III)	1.0×10^{-10} M
C5a	1.2×10^{-9} M
F-met-leu-phe	6.5×10^{-10} M
5-HETE	1.0×10^{-8} M

and macrophages (Smith, Ford-Hutchinson & Bray, 1980). LTB_4 should be considered as an important mediator of leucocyte movement.

We are grateful to Dr. T.J. Williams for supply of C5a and the Arthritis and Rheumatism Council for financial support (M.A.B.).

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Leukotriene B: A potent chemotactic agent and stimulus for lysosomal enzyme secretion for human neutrophils (PMN)

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Leukotriene B (LTB), a product of the lipoxygenase pathway of arachidonic acid metabolism (Borgeat & Samuelsson, 1979), is a potent chemokinetic, aggregatory and chemotactic agent for human PMN (Ford-Hutchinson, Bray, Doig, Shipley & Smith, 1980; Palmer, Stepney, Higgs & Eakins, 1980). Chemotactic agents such as the complement fraction C5a and the N-formylated peptides also induce the secretion of lysosomal enzymes from cytochalasin B (Cyt B) pretreated PMN *in vitro* (Becker, Showell, Henson & Hsu, 1974; Becker, 1976). We have analyzed the chemotactic activity of LTB and studied the release of lysosomal enzymes from CytB pretreated PMN *in vitro*.

Peripheral blood PMN were isolated and chemotaxis assayed in modified Boyden chambers as previously described (Palmer, Stepney, Higgs & Eakins, 1980). Chemotactic activity was analyzed in a checkerboard assay (Zigmond & Hirsch, 1973). For lysosomal enzyme secretion studies, 5×10^6 PMN in 1 ml HBSS were pretreated with Cyt B (5 μg/ml) for 15 min prior to addition of LTB (10^{-10} to 10^{-6} M) followed by a further 15 min incubation at 37°C. Incubation was terminated by centrifugation at 12,000 *g* for 30 s and the supernatant removed for assay of β-glucuronidase (Ringrose, Parr & McLaren, 1975) lysozyme (Smolelis & Hartsell, 1949) and lactate dehydrogenase (LDH) (Wroblewski & La Due, 1955).

LTB was found to be a potent chemotactic agent for human PMN with maximal activity at 10^{-9} M. This concentration produced a twofold increase over the control in the distance moved into the filters, whereas the synthetic peptide F-Met-Leu-Phe (FMLP) produced a 5–6 fold increase at the concentration that produced maximum chemotactic activity (3×10^{-10} M). Checkerboard analysis of the chemotactic response to LTB indicated that this agent is truly chemotactic as well as being chemokinetic.

CytB pretreated PMN exposed to LTB secreted both the azurophilic granule marker β-glucuronidase

and the specific granule marker lysozyme in a dose dependent manner between 10^{-9} and 10^{-6} M. The lysozyme dose-response curve was apparently biphasic, possibly reflecting sequential secretion from specific and azurophilic granules. The cytoplasmic marker LDH was not released. The ED_{50}'s for these responses for FMLP were 2×10^{-8} M and 6×10^{-9} M respectively with no release of LDH.

Thus LTB is a potent chemotactic and chemokinetic agent and induces the secretion of lysosomal enzymes from human PMN *in vitro*. These properties are common to other chemotactic agents, such as C5a and F-Met peptides, which are thought to be mediators of inflammation. LTB should therefore be considered equally with the above chemotactic agents as a potential inflammatory mediator.

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The actions of L-glutamate and related compounds on the central neurones of the Horse-shoe crab, *Limulus polyphemus*

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L-Glutamate is likely to be an excitatory neuromuscular transmitter throughout the arthropods (Beranek & Miller, 1968; Takeuchi & Takeuchi, 1964; Takeuchi, Onodera & Kawagoe, 1980; Usherwood, 1978) but there is less evidence for glutamate as a central transmitter in this phylum. L-Glutamate both excites and inhibits neurones from the central nervous system of *Limulus* (James & Walker, 1979). The present study examines these different responses to glutamate.

Experiments were performed on neurones from the abdominal ganglia of *Limulus*. Intracellular recordings were made using glass microelectrodes filled with molar acetate. These recordings were amplified and displayed using conventional electrophysiological techniques. Compounds were applied either iontophoretically or by addition directly to the bath (vol. 10 ml) containing the preparation. The equipotent molar ratio (EPMR) for each compound was determined by finding the number of nmoles which pro-L-glutamate. *Limulus* Ringer used contained (mM): NaCl 444; KCl 9; $CaCl_2$ 37; Tris-HCl buffer 15; pH 7.4.

L-Glutamate was tested on a random sample of 85 unidentified neurones, threshold concentration $2–10 \times 10^{-6}$ M, and produced either a depolarization (D), 39 cells, a hyperpolarization (H), 18 cells, or a biphasic H to D response, 12 cells. Sixteen cells did not respond to L-glutamate. Kainate, quisqualate, ibotenate, D-glutamate, L-aspartate and N-methyl-DL-aspartate were tested on both L-glutamate D and H cells ($n = > 4$) and their potencies compared to L-glutamate. On cells excited by L-glutamate, both kainate and quisqualate were more potent than L-glutamate, EPMRs (s.e. mean) being 0.063 ± 0.016 and

0.0028 ± 0.0008 respectively. Neither compound inhibited cells inhibited by L-glutamate. Kainate was virtually inactive on these cells while quisqualate had a potent excitatory effect. L-Aspartate was less active than L-glutamate on both H and D cells with an EPMR of 11.8 ± 4.0 and 6.8 ± 1.7 respectively. N-Methyl-DL-aspartate was inactive on both cell types.

On cells inhibited by L-glutamate, ibotenate was approximately equipotent with L-glutamate, EPMR being 0.96 ± 0.22, and on these cells D-glutamate was less potent than L-glutamate, EPMR being 16.1 ± 4.31. On cells excited by L-glutamate, ibotenate was also approximately equipotent with L-glutamate in producing excitation but the excitation was always preceded by an inhibitory phase. Similarly D-glutamate also caused inhibition on L-glutamate D cells. The inhibitory phases of the L-glutamate and ibotenate responses are chloride-mediated with a reversal potential of −60 mV and are reversibly blocked by picrotoxinin. *Limulus* neurones are inhibited by γ-aminobutyric acid (GABA) and this inhibition is chloride-mediated and blocked by picrotoxinin. It is possible that the inhibitory ibotenate response in cells only excited by L-glutamate is due to ibotenate activation of a GABA receptor. In conclusion, although ibotenate is equipotent on both glutamate responses, the responses to D-glutamate, kainate and quisqualate indicate differences between H and D glutamate receptors.

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Neuropharmacological evaluation of TRH analogues with modified proline residues

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Although TRH is reported to be widely distributed in the brain and to exhibit potent activity in several pharmacological tests predictive for antidepressant drugs, the clinical evaluation of the tripeptide's therapeutic potential has generally provided disappointing results. Such equivocal results may merely reflect the short biological half life of injected TRH so that a clearer indication of clinical utility would be obtained from a stabilised analogue of the endogenous tripeptide. Substitution of either a monomethyl (RX74355) or a di-methyl (RX77368) grouping into the proline residue at the 3 position resulted in molecules with increased resistance to enzymatic degradation. These have been evaluated in tests designed to probe the neuropharmacological properties of the molecules.

Using the reversal of reserpine-induced hypothermia (Askew, 1963) as an index of potential antidepressant activity RX74355 was 4 times more potent and RX77368 50 times more potent than TRH following i.v. administration. The peptides were also active after oral administration with an oral/i.v. ratio of about six. So called atypical antidepressants (e.g. mianserin) although not active in the reserpine reversal test, may be detected in the learned immobility test (Porsolt, Le Pichon & Jalfre, 1977). Both TRH and its analogues were active in this test and RX77368 exhibited enhanced potency when compared to either amitryptyline, mianserin or the parent tripeptide. The ability to antagonize clonidine-induced hypothermia after acute administration of a drug has been suggested to detect antidepressant drugs with a rapid onset of action (von Voigtlander, Triezenberg & Losey, 1978). Both RX74355 and RX77368 were active in this test and RX77368 was 10 times more potent than TRH.

Perhaps the most widely reported pharmacological effect of TRH is its ability to antagonize barbiturate sleeping time (Breese, Cott, Cooper, Prange, Lipton & Plotnikoff, 1975). RX74355 was 33 times more potent and RX77368 220 times more potent than TRH in such a test. This last result suggests that TRH peptides may have CNS stimulant properties rather than a classical antidepressant profile. Direct measures of cortical EEG in unrestrained rats confirmed a stimulant action for the peptides. However, such 'stimulation' was not accompanied by the behavioural stimulation (e.g. increased locomotor activity) associated with psychic stimulants such as amphetamine.

Thus the stabilized analogues of TRH exhibit enhanced potency in animal tests considered predictive for antidepressant activity. However, the pharmacological profiles of the analogues are unusual and do not correspond to the profiles of other types of CNS drugs. Human evaluation will be necessary to confirm the therapeutic potential for such molecules.

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Effect of 3,5-dimethylpyrazole on mouse brain catecholamine levels

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Pyrazole, when administered to mice, causes a dose-dependent decrease in brain noradrenaline (NA) levels (MacDonald, 1976). This we have attributed to an *in vivo* inhibition of dopamine-β-hydroxylase (DBH) activity. We have attempted to obtain a derivative of pyrazole which would i) cause a rapid dose-dependent decrease in central NA levels, ii) inhibit DBH *in vivo* and *in vitro* and iii) be less toxic than pyrazole. From a series of methylated pyrazoles, we chose 3,5-dimethylpyrazole (3,5-DMP) for further study.

Three h after administration (i.p.) of 3,5-DMP to adult male mice, brain NA levels were maximally reduced by up to 50% compared to saline treated mice. Doses greater than 5 mmol/kg (480 mg/kg) had no further effect and doses lower than 2 mmol/kg were ineffective. The decline in brain NA levels was accompanied by a slight increase in brain dopamine (DA) concentrations and a substantial increase (over 50%) in the levels of the metabolites of dopamine, homovanillic acid and 3,4-dihydroxyphenylacetic acid.

3,5-DMP had no effect of DBH activity *in vitro* but a 30% decrease in activity in rat hypothalamus was obtained 6 h after 3 mmol/kg. Since pyrazoles can interfere with the assay procedure for DBH (Brown & Harralson, 1976), we have assessed DBH activity also by studying the changes in brain catecholamine levels after treatment of mice with pargyline (100 mg/kg) and L-DOPA (30 mg/kg). 3,5-DMP treated mice, while showing an increase in brain NA levels after pargyline showed no further increase after subsequent L-DOPA treatment. This result was duplicated if the rats were previously reserpinised to prevent feedback inhibition of synthesis. Dopamine levels were usually relatively higher in 3,5-DMP mice.

3,5-DMP at doses greater than 3 mmol/kg caused sedation and the highest dose tested, 9 mmol/kg was anaesthetic. Mice treated for 30 days with 3,5-DMP (5 mmol/kg) showed no signs of toxicity. Some tolerance developed to the sedatory effect and the NA-depleting effect was somewhat inhibited.

It is concluded that 3,5-DMP possesses similar central effects as pyrazole but it is much less toxic than the parent molecule. We consider that the depletion of brain NA is due to an inhibition of DBH and preliminary results reveal that a metabolite of 3,5-DMP, 3-carboxy-5-methylpyrazole is able to inhibit DBH *in vitro*.

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Lack of effect of μ and κ opiate receptor agonists on K^+-stimulated release of Met-enkephalin from guinea-pig striatal slices

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In common with other neurotransmitter systems it is possible that the release of endogenous enkephalin from peptidergic neurones is controlled by a presynaptic opiate receptor. Previous studies (e.g. Richter, Wesche & Frederickson, 1979) have shown that neither morphine nor naloxone affected the K^+ evoked release of Met^5- and Leu^5-enkephalin from rat isolated striatal slices. However, both of these drugs act predominantly on only one of the sub-types of opiate receptor, i.e. the μ receptor. The heterogeneity of opiate receptors is well recognised (Martin, Eades, Thompson, Huppler & Gilbert, 1976; Tyers, 1980) and therefore before discounting an opioid autoinhibitory mechanism, it is important to determine the effects on release of interactions with other receptor subtypes. The present study was carried out to determine whether interaction with the κ-opiate receptor affected Met^5 enkephalin release from guinea-pig isolated striatal slices.

Male guinea-pigs, weight range 250–300 g, were killed by cervical dislocation and exsanguination. The brain was rapidly removed and placed in ice cold Krebs solution. Striatal slices (300 μm) dissected from the whole brain were prepared using a McIlwain tissue chopper. The slices were separated and suspended in Krebs soluton at 37°C and gassed with 95% O_2 and 5% CO_2. After a 30 min equilibration period the slices were superfused with Krebs solution at 1 ml/min and the superfusate collected in plastic vials on ice for three 5 min periods. The Krebs solution was changed for one in which K^+, 5×10^{-2} M, replaced an equivalent amount of Na^+ and a further three collections of superfusate made. The effects of morphine, [(−)-α-(1R, 5R, 9R)-S,9-dimethyl-2-(L-tetrahydrofurfuryl)-2'-hydroxy-6,7-benzomorphane] (Mr 2034), a κ opiate receptor agonist, and naloxone, all at 1×10^{-6}M on the K^+ evoked release of Met^5 enkephalin were determined by adding these drugs to the superfusing medium throughout the whole experiment. Calcium dependency studies were carried out using striatal slices which were superfused with Krebs containing Ca^{2+}, 2.54×10^{-6} M for the first three collections. The superfusing medium was then changed for the high K^+ medium containing no Ca^{2+}. Three further superfusate collections were made. At the end of each experiment the tissue was homogenised, centrifuged and the supernatant with samples, stored frozen until assay. Their Met^5 enkephalin content was determined using a radioimmunoassay (Immunonuclear Corp., Minnesota, USA; 0.09 ng/ml sensitivity).

High K^+ concentration caused a mean 6.7 fold increase in enkephalin release. (Basal levels < 0.09 ng/100 mg tissue) which was calcium dependent. Neither morphine, naloxone nor Mr. 2034 (6.9, 8.7 and 8.5 fold increases respectively) significantly affected the K^+ stimulated release of enkephalin.

It is concluded that if presynaptic autoinhibitory receptors exist for endogenous enkephalin release then these are not of the μ- or κ-type. Enkephalin interacts selectively *in vitro* with the δ receptor which has not been examined in this study and remains as a possible autoinhibitory receptor candidate.

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Release of endogenous dopamine from the neuro-intermediate lobe of the rat hypophysis *in vitro*

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The neural and intermediate lobes (N.I.L.) of the hypophysis are innervated by dopaminergic nerves (Björklund, 1968). A connection between hormone secretion from these lobes and dopamine (DA) was suggested by the observation that in lactating rats the DA content of the N.I.L. was decreased and the DA turnover increased (Holzbauer, Sharman & Godden, 1978a) and also by the rise in the pituitary DA content (Holzbauer, Sharman & Godden, 1978b; Torda, Lichardus, Kvetňanský & Ponec, 1978) and the increase in synthesis (Alper, Demarest & Moore, 1980) after dehydration and NaCl loading.

The present report describes the release of endogenous DA from the N.I.L. Rats were injected with pargyline (50 mg/kg b.wt. i.p.) 1 h before decapitation. The N.I.L. was dissected out and preincubated in Ca^{2+}-free Krebs-bicarbonate with glucose and pargyline (1 μM) for 15 minutes. Four N.I.L. were then transferred to a microbath containing 40 μl of normal Krebs-bicarbonate (Ca^{2+}: 2.5 mM; K^+: 4.7 mM) at 37°C. After 10 min the incubation medium was collected and 40 μl of potassium enriched (47.0 mM) Krebs-bicarbonate was added to the glands. The incubation was continued for another 10 min and the medium again collected. The catecholamine content of each incubation medium was estimated by high performance liquid chromatography with electrochemical detection (Keller, Oke, Mefford & Adams, 1976; Bennett, Marsden, Sharp & Stolz, 1980).

A basal release of DA from one N.I.L. of the order of 350 femtomoles in 10 min was observed. The basal release of noradrenaline (NA) from one N.I.L. was about 200 femtomoles in 10 minutes. A ten-fold increase in the K^+-concentration of the incubation medium caused a four-fold increase in the release of DA, the amount released being equivalent to about 10% of the amount contained in the N.I.L. of an untreated rat. Simultaneously there occurred a two-fold increase in the release of NA. GABA caused an increase in the release of DA from the medial basal hypothalamus but not from the N.I.L.

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Drug-induced alterations of the post-ictal seizure threshold increase

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Following an electrically induced seizure a greater stimulus is required to elicit a subsequent seizure (Herberg, Treis & Blundell, 1969). We have shown that immediately following an electroconvulsive shock (ECS) there is an increased seizure threshold to the GABA antagonist drugs pentylenetetrazol (PTZ), bicuculline and isopropylbicyclophosphamate, but not to the glycine antagonist, strychnine. The increased threshold still occurred when animals were pretreated with α-methyl-*p*-tyrosine, *p*-chlorophenylalanine, naloxone, or indomethacin (unpublished observations).

Table 1 Effects of pretreatment with various drugs on the increase in seizure threshold seen after electroconvulsive shock (ECS)

Drug		Pretreatment time (min)	Seizure threshold following Sham shock	ECS	P Value
Saline		5	23 ± 2 (4)	36 ± 5 (5)	< 0.005
Bicuculline	3 mg/kg	5	27 ± 2 (5)	33 ± 3 (5)	< 0.001
PTZ	30 mg/kg	5	20 ± 4 (4)	43 ± 5 (4)	< 0.001
Saline		5	29 ± 4 (9)	38 ± 4 (10)	< 0.001
Diazepam	2 mg/kg	5	47 ± 14 (5)	72 ± 20 (6)	< 0.05
Diazepam vehicle		5	32 ± 3 (4)	43 ± 7 (5)	< 0.05
Flurazepam	10 mg/kg	5	44 ± 5 (4)	64 ± 12 (6)	< 0.02
Saline		30	23 ± 2 (5)	35 ± 2 (5)	< 0.001
(+)-Propranolol	40 mg/kg	30	22 ± 2 (5)	25 ± 4 (5)	n.s.
Saline		30	36 ± 5 (6)	49 ± 6 (6)	< 0.005
(−)-Propranolol	40 mg/kg	30	32 ± 3 (4)	31 ± 3 (5)	n.s.
Saline		30	30 ± 3 (5)	40 ± 6 (5)	< 0.02
Timolol	40 mg/kg	30	29 ± 3 (5)	39 ± 4 (4)	< 0.001
(−)-Propranolol	1 mg/kg	30	34 ± 3 (8)	41 ± 3 (5)	< 0.002
Phenytoin	40 mg/kg	30	33 ± 4 (9)	40 ± 5 (9)	< 0.001
Phenytoin	80 mg/kg	30	30 ± 5 (5)	37 ± 2 (5)	< 0.01
Lignocaine	20 mg/kg	15	40 ± 2 (5)	45 ± 4 (6)	< 0.05
Lignocaine	40 mg/kg	15	57 ± 5 (4)	52 ± 11 (4)	n.s.

Seizure thresholds are expressed as mean ± s.d. in mg/kg of PTZ. Number of animals in each group shown in brackets. Seizure thresholds were determined 30 min after ECS or sham. Horizontal lines separate groups of experiments performed with their respective saline controls. All drugs were administered as solution in 0.9% w.v. saline except diazepam and phenytoin which were administered as the commercially available injectable solution. Pretreatment time refers to the period between drug injection and ECS/sham shock. All statistics analysed by Student's *t*-test.

We have now investigated the effects of other drugs on the rise in seizure threshold following an ECS.

Male Sprague-Dawley rats (175–200 g) were used. Seizure thresholds were determined using a timed infusion of PTZ (concentration 10 mg/ml in 0.9% saline) until the onset of myoclonic jerks (for details see Nutt, Cowen & Green, 1980). A single ECS (125 v, 1 s) was administered through earclip electrodes and seizure thresholds were determined 30 min later. To allow for variation in the seizure thresholds of untreated animals (Frey & Löscher, 1980), each set of experiments included a control group.

Pretreatment of animals with subconvulsive doses of PTZ or bicuculline failed to block the rise in seizure threshold following an ECS (Table 1). Diazepam and flurazepam both elevated basal seizure threshold, and ECS produced a further significant rise.

The effect of agents reported to block maximal electroshock seizures and also (+)-propranolol which exhibits a similar action (unpublished observations) were investigated. Lignocaine appeared to prevent the ECS-induced increase in threshold but also elevated the basal seizure threshold. Phenytoin was without effect on basal seizure threshold, or the ECS-induced increase. (+)-propranolol had no effect on basal seizure threshold but abolished the ECS-induced rise. (−)-propranolol and timolol in β-adrenoceptor antagonist doses did not prevent the ECS-induced increase.

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Brain tryptophan and 5-HT function in stress

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Restraint stress has been shown to increase 5-hydroxytryptamine (5-HT) turnover as measured by increased 5-hydroxyindoleacetic acid (5-HIAA), the metabolic product of 5-HT, in the absence of changes in the latter (Bliss, Ailion & Zwanziger, 1968). Brain tryptophan, the amino acid precursor of 5-HT, has also been shown to increase specifically (Knott, Joseph & Curzon, 1973). However it has been suggested that increased 5-HIAA may not reflect increased functional 5-HT release and merely indicates increased 5-HT metabolism (Grahame-Smith, 1971). To investigate this possibility we have studied the effect of blocking the increase of tryptophan on the metabolic and functional 5-HT response to stress. This was achieved by pretreatment with valine, a neutral amino acid which competes with tryptophan for uptake into the brain (Jacoby, Thomas, Poulakos & Siegel, 1979). As an index of 5-HT functional activity, in the hypothalamus at least, plasma corticosterone was measured, as corticotrophin releasing factor (CRF) release from the hypothalamus is at least partially dependent on 5-HT (Buckingham & Hodges, 1979).

Male Sprague Dawley rats, 200–280 g in groups of 9, were injected with isotonic saline or valine 200 mg/kg in isotonic saline. Restraint stress was achieved by tying to a wire grid (Knott, Joseph & Curzon, 1973). After 1 or 2 h the rats were decapitated and blood and brains collected. Plasma tryptophan, brain tryptophan, 5-HT & 5-HIAA were determined fluorimetrically as previously described (Joseph, 1978) as was corticosterone (Mattingly, 1962). The experiments showed that the rise in brain tryptophan induced by restraint stress in rats (60.2% at 1 h and 39.7% at 2 h $P < 0.001$) was prevented by administration of valine 200 mg/kg i.p. 1 h previously, (-12% and -19% after 1 or 2 h of stress respectively). Brain 5-HT was not depleted but the stress induced rise in 5-HIAA (34.7% at 1 h, 38.5% at 2 h $P < 0.001$) was prevented ($+5\%$ at 1 h, $+20\%$ at 2 h $P < 0.001$ and $P < 0.005$ significantly different from stress at 1 and 2 h values respectively). The 5-HT mediated functional response to stress, elevated plasma corticosterone was however also significantly attenuated by valine pretreatment from an increase of 343% at 1 h and 354% at 2 h $P < 0.001$ to 57% and 40% of these increases respectively $P < 0.001$). Valine treatment alone in the absence of stress however had little effect on corticosterone which argues against a mode of action independent of 5-HT. This suggests that the brain 5-HIAA increase after stress is not merely secondary to increases in brain tryptophan but does indicate an increase in functional 5-HT activity which is in turn at least partly dependent on the increase in brain tryptophan.

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Behavioural and biochemical changes following withdrawal of chronic dexamphetamine or bupropion administration in rats

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Depression has been reported in both rats and man following withdrawal from chronic treatment with dexamphetamine (Tonge, 1974; Schildkraut, Watson, Draskoczy & Hartmann, 1971). We have now studied the effects on locomotor activity and on brain noradrenaline and dopamine stimulated adenylate cyclase in rats after withdrawal from chronic treatment with dexamphetamine or bupropion, a new antidepressant known to possess some dexamphetamine-like properties in rats (Soroko, Mehta, Maxwell, Ferris & Schroeder, 1977).

Male Wistar hooded rats (initial weight 100–120 gm, $n = 6$ or 12 per treatment) received the drugs in drinking water in increasing concentrations over 21 days; bupropion from 1 to 4 g/litre and dexamphetamine from 50 to 200 mg/litre. An equal weight of ascorbic acid was added to the dexamphetamine solutions as an antioxidant (Lynch & Leonard, 1978). The average maximum drug consumption dexamphetamine and bupropion were 17 and 300 mg/kg per day respectively.

On withdrawal from dexamphetamine nocturnal locomotor activity, as measured by ultrasonic activity monitoring equipment (6 channel; C.F. Palmer, High Wycombe), for the period 17.00 to 03.00 (rats maintained on 12 h dark/light cycle; lights off from 17.00 to 05.00), was significantly reduced ($P < 0.01$), for Days 1 and 2 (46% and 73% of control values respectively) returning to control levels on Day 3. Rats withdrawn from bupropion had significantly decreased activity ($P < 0.05$) on Days 1 and 3 (67% and 60% of control values respectively) but not on Day 2 after withdrawal. The cyclic changes in activity after bupropion withdrawal are similar to the changes after dexamphetamine reported by Tonge who reported a temporary return to normal activity at 24 hours.

The sensitivities of the limbic noradrenaline stimulated adenylate cyclase preparation (Blumberg, Vetulani, Stamwarz & Sulser, 1976) and striatal dopamine sensitive adenylate cyclase preparation (Kebabian, Petzhold & Greengard, 1972) were measured for days 1 and 2 of withdrawal of drug treatment. The values in the dexamphetamine-treated groups were significantly altered: noradrenaline stimulated cAMP production was increased (+90%; $P < 0.05$) as also was the dopamine stimulated adenylate cyclase (+60%; $P < 0.05$). In the bupropion treated rats no significant changes were found in the cyclase preparations.

Although both bupropion and dexamphetamine produce similar behavioural changes after withdrawal they apparently differ with regard to their biochemical effects as had previously been suggested by studies on amine uptake in rat brain *in vivo* (Canning, Goff, Leach, Miller, Tateson & Wheatley, 1979).

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Selective enhancement of dopamine (induced behaviours) following chronic neuroleptic treatment

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Behavioural responses produced by dopamine-like agonists are enhanced following long term treatment with neuroleptic compounds. This observation has been ascribed to the development of dopaminergic receptor supersensitivity (Muller & Seeman, 1978). For example, increased stereotyped responses to apomorphine have followed treatment with haloperidol, loxapine and chloropromazine although no change was observed after clozapine (Sayers *et al.*, 1975). This latter effect was said to reflect the low incidence of tardive dyskinesias associated with the clinical usage of clozapine. However other workers have demonstrated behavioural supersensitivity following clozapine adminstration (Smith & Davis, 1976). In the above studies the dopamine-like agonists were administered peripherally. In an attempt to resolve these conflicting findings we have examined the effects of long term treatment with haloperidol, clozapine and metaclopramide on behavioural responses produced by intracerebral injections of dopamine. Metoclopramide was included in this study because of its poor antipsychotic activity and failure to antagonise the effect of intraaccumbens dopamine when administered peripherally (Costall & Naylor, 1976).

Male Wistar rats (Charles River) weighing 130–150 g at the start of the experiment were dosed orally (5 ml/kg) for 22 days with either haloperidol (3 mg/kg), clozapine (60 mg/kg), metoclopramide hydrochloride (30 mg/kg) or CMC vehicle. On termination of dosing (day 23) animals were implanted with cannulae for direct injections into either the nucleus accumbens (NAS, A + 9.4, L ± 1.6, VO) or caudate (A + 8.0, L ± 3.0, V + 1.0; Pellegrino & Cushman, 1967) using standard stereotaxic techniques. Four days later (Day 27) locomotor activity was measured following bilateral dopamine (2.5 μg) injections into the nucleus accumbens. Animals with caudate cannulae received (10 μg bilaterally) and were scored for stereotyped behaviour according to the method of Costall & Naylor (1977). All the animals received nialamide 50 mg/kg i.p. 2 h prior to the dopamine injections.

All three treatments produced a significant enhancement of dopamine induced locomotor activity in the NAS group ($P < 0.001$ Kruskal Wallis); haloperidol and metoclopramide treatment producing a significant increase within 1 h ($P < 0.01$ and $P < 0.05$ Mann Whitney) while clozapine enhanced the activity within 2 h ($P < 0.01$). Following intracaudate dopamine, licking and biting behaviour was observed in the haloperidol and metoclopramide treated groups ($P < 0.001$ and $P < 0.01$ respectively compared to controls, Mann Whitney) whereas mild sniffing was observed in the control and clozapine treated groups. These findings are consistent with the hypothesis of receptor supersensitivity following long term neuroleptic treatment. However the behavioural correlates are differentially affected according to treatment. This suggests that this method may be useful in assessing the relative liability of a compound to cause tardive dyskinesia.

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Dopaminergic stereotyped and turning behaviour in the rabbit

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Dopamine release by drugs has mostly been studied in rodents. Only three papers, as far as we know, have dealt with dopaminergic activity in rabbits (Harnack, 1874; Bertler & Rosengren, 1959; Ernst, 1965.)

Three well known dopamine-like drugs were given to rabbits at random by i.v. route (methamphetamine and amphetamine) or i.m. route (apomorphine). Observation time was 60 minutes. Stereotyped behaviour included (i) foot-shock, concentration with sudden rise of hind limbs, followed by a knock on the metal floor of the cage (which facilitates the counting) (ii) rapid turning behaviour, by at least 180° (iii) repetitive licking or gnawing of cage. The licking and gnawing behaviour was less constant, irregular, difficult to measure and not proportional to dose.

Behaviour patterns (i) and (ii) were used for screening. Strain and sex variations were studied in three types of rabbits: fulvous rabbit of Burgundy (Table 1), albino rabbit and domestic rabbit. The sensitivity to dopaminergic drugs was constant in Burgundy rabbits, variable in domestic rabbits and low in albino rabbits (about 25% reactive animals). Sex variations were much less apparent: females were slightly more sensitive than males.

In rabbits, apomorphine produced almost exclusively stereotyped behaviour while the analeptics produced more turning behaviour (both substances being active at similar dose levels).

Methamphetamine was more active than amphetamine and would be preferred as a test substance. Stereotypies and turning behaviour diminished when these two drugs were given again 24 h later. A period of at least five days was necessary for full recovery of sensitivity.

Three antagonists (pimozide, metopimazine, supiride) were investigated and it was possible to obtain selective inhibition of foot shock phenomenon or turning behaviour. Thus, it would seem to be possible to differentiate the effects of sympathomimetic drugs as being more like dopamine or more like noradrenaline.

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Table 1 Results obtained with apomorphine, amphetamine and methamphetamine in 3 batches of 12 female Burgundy rabbits

	Dose	*Stereotypies**	*Turnings**
Apomorphine	5 μM/kg	80.67 ± 10.05	0.86 ± 0.23
Amphetamine	50 μM/kg	17.00 ± 5.67	78.89 ± 16.30
Methamphetamine	50 μM/kg	18.13 ± 2.97	138.33 ± 16.01

* mean ± s.e. mean.

Pharmacological manipulations of tectal GABA systems affect posture and locomotion in the rat

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Current evidence suggests there is a major GABAergic projection from the substantia nigra zona reticulata (SNR) to the superior colliculus (SC; Di Chiara, Porceddu, Morelli, Mulas & Gessa, 1979; Vincent, Hattori & McGeer, 1978). Bilateral injections of muscimol into the SNR (Scheel-Krüger, Arnt & Magelund, 1977), or of picrotoxin into the SC (Dean, Lewis, Redgrave & Souki, 1980) reportedly elicit hypermotility and stereotypy, indicating the nigrotectal pathway may be an important route via which control of these behaviours is exercised. We have further explored this possibility by determining the effects of drugs injected into the SC on the circling behaviours elicited by unilateral application of muscimol or kainic acid to the SNR. Stereotaxic injections were delivered in 0.2 µl volume over 3 min to albino Wistar rats anaesthetized with halothane (1.5% in oxygen) using a 1 µl Hamilton syringe.

Unilateral injection of picrotoxin (40 ng, $n = 16$) or tetanus toxin (TT; 30 mouse lethal doses, $n = 22$) into lateral (L 1.5–2.0, A 0.0–1.7; König & Klippel, 1963) regions of intermediate or deep layers of the SC evoked tight contraversive circling and stereotyped gnawing. Similar injections ($n = 12$) made into the medial aspects (L 0.5–1.0) of the same tectal layers elicited explosive motor behaviour (EMB) characterized by episodes of violent leaping and forward running. In TT-treated rats this EMB progressed to tight contralaterally-directed spiralling after 4–6 hours. The picrotoxin effects were short-lived (40 min). Unilateral injection of muscimol (40 ng) into either medial ($n = 8$) or lateral ($n = 12$) parts of intermediate or deep tectal layers provoked short-lasting (10 min) ipsiversive circling and gnawing; similar injections given bilaterally ($n = 6$) caused rats to gnaw and to adopt a 'frozen' posture for up to 60 min, but did not produce rigid catalepsy (see Di Chiara, Morelli, Porceddu & Gessa, 1979). It is noteworthy that each of the different tectal regions tested in the behavioural study exhibited a significant reduction (19–33%, $P < 0.005$, $n = 8$) in GABA content following electrolytic lesion of the ipsilateral SNR, consistent with the widespread innervation of the tectum by GABAergic projection neurones.

Injecting one SNR with muscimol (40 ng, A 1.6, L 2.0, V −2.4) evoked tight contraversive circling having an average peak frequency of 19.4 turns/min ($n = 10$). This response was significantly attenuated by the following procedures: 1). a 5 day-old kainic acid (1 µg) lesion of the ipsilateral (53% reduction in peak turning rate, $P < 0.001$, $n = 9$) or contralateral tectum (28% reduction, $P < 0.001$, $n = 9$), and 2). prior injection of muscimol (40 ng) unilaterally into the ipsilateral tectum (76% reduction, $P < 0.001$, $n = 10$), or bilaterally into both tecta (52% reduction, $P < 0.001$, $n = 10$).

Conversely unilateral injection of kainic acid (1 µg) gave rise to acute ipsiversive circling interspersed with running seizures. The kainate-induced asymmetry, but not the hyperactivity, was prevented by injecting the ipsilateral tectum with TT 24 h earlier. The postural asymmetry produced by the TT treatment itself, however, was unchanged. Considered together the present results suggest an involvement of tectal GABAergic mechanisms in the expression of nigral-derived postural and locomotor asymmetries.

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Studies on benfluorex and its metabolites on glucose uptake into rat isolated hemidiaphragm

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The trifluormethyl compound fenfluramine sigificantly enhances glucose uptake into rat and human skeletal muscle (Turner, 1978). A related compound benfluorex (780SE) has hypolipidaemic and hypoglycaemic properties in man (Asmal & Leary, 1975; Glikmanas, Giraudet & Fould, 1979). We have therefore studied the influence of benfluorex and its metabolites, norfenfluramine, S422, S1475 and S1513 in the rat hemidiaphragm model. Benfluorex (12.5–100 ng/ml) had a biphasic action, concentrations of 12.5–25 ng/ml increased glucose uptake significantly, but 50–100 ng/ml caused a dose related inhibition. Norfenfluramine increased glucose uptake at a concentration of 100 ng/ml, the magnitude of the increase being inversely correlated to basal glucose uptake levels. The metabolite S1475 facilitated glucose uptake at a concentration of 100 ng/ml. Neither S422 nor S1513 had any effect on glucose uptake at concentrations of 50–200 ng/ml.

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Investigations into the suitability of the male rat as a source of hepatocytes for studies of drug interactions which may involve induction of acetylation

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Drug interactions can occur by a variety of mechanisms (McElnay & D'Arcy, 1980). *In vitro* simulations of these mechanisms can be useful in predicting the extent to which drugs may be involved in such interactions.

As an *in vitro* tool isolated hepatocytes have been used to investigate drug metabolism e.g. acetylation of sulphonamides and isoniazid (Morland & Olsen, 1977), and also the effect of inducing agents such as phenobarbitone and 3-methylcholanthrene on different substrates (Thor *et al.*, 1978). They have seldom been used specifically to study drug interactions.

Despite reports of induction of sulphadiazine acetylation in spironolactone pre-treated female rats (Kourounakis & Selye, 1976), this metabolic route has not previously been implicated in drug interactions. Acetylation induction, however, could provide an alternative explanation to that proposed by Mitchell *et al.*, (1975) for isoniazid-rifampicin hepatotoxicity (Pessayre *et al.*, (1977)). Prior to *in vitro* investigation of this and similar interactions, some *in vivo* studies were undertaken to assess the suitability of the rat for such experiments (Experimental details are given in Table 1).

Following recovery of sulphadimidine and N_4-acetylsulphadimidine from urine, using a modified Wooley & Sigel (1978) technique, quantification was achieved by reversed-phase HPLC with UV detection at 270 nm (modified from Bagon, 1979).

The results from this work (Table 1), showed that neither rifampicin nor spironolactone had a significant inductive effect upon acetylation of sulphadimidine.

In addition, no significant increase in total urinary excretion of sulphadimidine or N_4-acetylsulphadimidine was found, contrary to the report of Kourounakis & Selye (1976) ($P > 0.05$).

Due to the absence of the acetylation induction by rifampicin or spironolactone in this study, male rats may not be suitable for *in vitro* studies of drug interactions involving possible induction of acetylation.

Table 1 Three groups of five male rats (Wistar, 140 g–220 g) were used, one group served as control, the others being treated with either spironolactone (100 mg/kg i.p.) or rifampicin (50 mg/kg i.p.) twice daily for three days. On day five, sulphadimidine (40 mg/kg i.p.) was administered and urine collected for 24 hours. Each group of drug-treated rats was used twice and the control group was used on four occasions. The time interval between the duplicate drug treatments was ten days.

	$Ratio \frac{N_4\text{-acetylsulphadimidine}}{\text{sulphadimidine}}$	
Rifampicin treated	1.81 ± 0.17 $n = 10$	$P > 0.05$
Control	1.72 ± 0.18 $n = 10$	
Spironolactone treated	2.08 ± 0.14 $n = 10$	$P > 0.05$
Control	1.89 ± 0.19 $n = 10$	

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Alteration of liver blood flow and metabolism on lignocaine and tocainide kinetics; observed changes and predictions of pharmacokinetic models

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Liver blood flow (LBF), enzyme activity and drug binding are major determinants of hepatic drug elimination. Two mathematical models have evolved to predict the influences of these variables on hepatic drug extraction, namely the 'well stirred' and the 'parallel tube' models (Pang & Rowland, 1977).

In previous communications we have described changes which occur in the kinetics of lignocaine (hepatic extraction (E) = 0.98 and tocainide (E = 0.06) in male CFY rats (a) when LBF was reduced by sotalol (Bennett, Notarianni & Supradist, 1980a) (b) when metabolism was increased by 34 benzpyrene (34BP) or (c) when both LBF and metabolism were increased by phenobarbitone (PB) (Bennett, Supradist & Notarianni, 1980b). The experimentally observed changes have now been compared to predictions of the above mathematical models.

All values are expressed as ml min^{-1} 100 $g.b.w.^{-1}$. LBF measured by radioactively labelled microspheres (McDevitt & Nies, 1976) was 4.06 ± 0.43 (mean $\pm$ s.e. mean) ($n = 10$) in control rats, 3.14 ± 0.34 ($P < 0.05$) ($n = 7$) after sotalol, 4.6 ± 0.34 ($n = 6$) after 34BP and 5.67 ± 0.56 ($P < 0.02$) ($n = 7$) after PB. Intrinsic hepatic clearance (CL_{int}), which is in-

dicative of drug metabolic activity, calculated for the 'well stirred' model for lignocaine was 73.0 ± 8.0 ($n = 8$) in control rats, 113.9 ± 13.0 ($P < 0.02$) ($n = 6$) after 34BP and 254.9 ± 13.9 ($P < 0.001$) ($n = 7$) after PB. CL_{int} for tocainide was 1.3 ± 0.2 ($n = 6$) in control rats, 2.4 ± 0.2 ($P < 0.01$) ($n = 6$) after 34BP and 2.9 ± 0.3 ($P < 0.01$) ($n = 6$) after PB.

After 34BP, for both models, predicted values for E, systematic availability and area under the blood concentration-time curve after i.v. and p.o. administration were similar to those observed for both lignocaine and tocainide. After sotalol the predicted values for these parameters for tocainide were in good agreement with those which were observed. The greatest difference between observed and predicted values with both models was found in the kinetics of the blood-flow dependent drug lignocaine when hepatic blood flow was changed.

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The development of a radioimmunoassay for ranitidine in biological fluids

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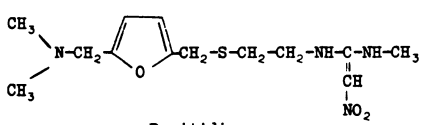
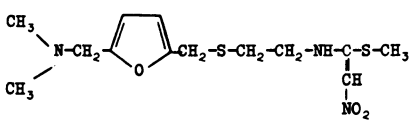
The h.p.l.c. method (Carey & Martin, 1979) for determining ranitidine, an H_2 receptor antagonist, in plasma is time consuming, and in order to study the pharmacokinetics of ranitidine in man, a more rapid radioimmunoassay has been developed. It was not possible to conjugate ranitidine with a suitable protein but it was possible to conjugate the thioether analogue of ranitidine to the ε aminolysyl groups of bovine albumin. Six molecules of the hapten were conjugated per mole of protein (Figure 1). The conjugate was emulsified in Freunds complete adjuvant and injected into New Zealand white rabbits. An antiserum was produced in each rabbit. Using a final

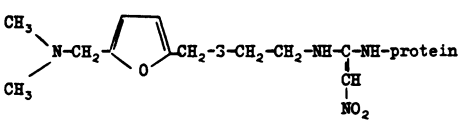
SCHEME 1



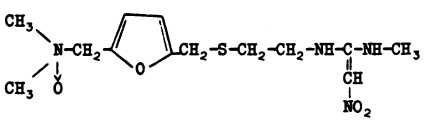
Ranitidine



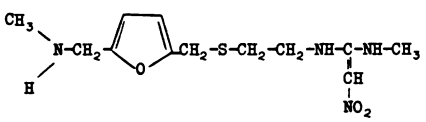
S-methyl analogue of ranitidine



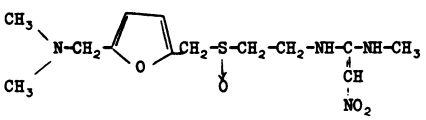
Protein conjugate



Metabolite I



Metabolite II



Metabolite III

dilution of antiserum of 1 in 1250 [^{3}H]-ranitidine was displaced from the antibody binding site by standard solutions of ranitidine. A standard curve was constructed which was sensitive to 1 ng ranitidine/ml. The mean recoveries of ranitidine from normal serum was 95 ± 6% over the concentration range 2–2000 ng/ml. The coefficient of variation of the between assay measurements of serum ranitidine from volunteers receiving the drug was 7%. Metabolites I, II and III (Bell, Dallas, Jenner & Martin, 1980, Figure 1) were shown to have a cross reactivity with the antisera of < 0.1, 11 and 22% respectively. The percentage of the dose excreted in the urine as I, II and III corresponds respectively to 5, 2 and 1% (Carey, Martin & Owen (1980) in press). The specificity of the assay was checked by determining the ranitidine concentration of the same plasma samples using the radioimmunoassay and the h.p.l.c. method. The correlation coefficient between the two assays was 0.98. These results indicate that the concentration of circulating metabolites II and III in the plasma were too low to interfere in the radioimmunoassay of ranitidine. The assay has been used to determine ranitidine in human breast milk, CSF and urine.

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Pharmacokinetics and bioavailability of ranitidine in humans

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The pharmacokinetics of intravenous and oral doses of ranitidine have been studied in 12 fasted volunteers, Table 1. Plasma ranitidine concentration was determined by radioimmunoassay (Fellows, Jenner, Martin & Willoughby, 1980). The plasma concentration-time curves after oral administration showed in some cases two peaks and in others a flat plasma concentration profile for up to 3 hours. No such second peak in the plasma concentration curve was seen after intravenous injection. In a separate study in volunteers who received food after having an oral dose of ranitidine (150 mg) there was no second peak in the plasma concentration-time curves. A similar

Table 1 Pharmacokinetics of ranitidine in man

	i.v. dose (mg)	*oral dose (mg)*	
Parameters			
	100	100	150
$T_1\beta$ min	127 (7)	179 (24)	187 (37)
AUC 0–∞ ng ml^{-1} h^{-1}	2970 (380)	1680 (360)	2338 (560)
Cl (ml/min)	568 (61)	—	—
Vd (l/kg)	1.16 (0.09)	—	—
K_{12} (min^{-1})	0.092 (0.020)	—	—
K_{21} (min^{-1})	0.022 (0.003)	—	—
K_{el} (min^{-1})	0.037 (0.006)	—	—
Bioavailability (%)	—	56 (10)	52 (13)

Data calculated using the NONLIN programme (Metzler, 1976) The values are the mean of twelve subjects and the figures in parenthesis denote the standard deviation. AUC = area under plasma concentration–time curve, Cl = plasma clearance, Vd = apparent volume of distribution, K_{12}, K_{21}, K_{el} = transfer constants for a two compartment model.

phenomenon has been observed in patients treated with cimetidine (Somogyi, Rohner & Gugler, 1980). Pedersen & Miller (1980) subjected the cimetidine data to pharmacokinetic analysis and came to the conclusions (1) that the drug might accumulate in the hepatic parenchymal system or the bile, (2) the rate of accumulation is much higher in the first-pass transfer than from the systemic circulation, (3) absorbed elements of food compete with cimetidine and (4) when the fasted subjects were given food this could cause release of the drug from a storage depot. Only the plasma concentration-time data from the intravenous dose of ranitidine fitted the biexponential function $Ae^{-\alpha t} + Be^{-\beta t}$. The $T_{\frac{1}{2}}\beta$ after the 100 mg intravenous dose was 127 ± 7 min compared with 179 ± 24 min after the 100 mg oral dose and 187 ± 37 min after the 150 mg oral dose. The increased $T_{\frac{1}{2}}\beta$ of ranitidine after oral administration could be due to release of ranitidine from a storage depot, or recycling of the drug. The bioavailability of ranitidine based on the area under the curve method was about 50% after both the 100 and 150 mg oral dose. The renal clearance of ranitidine, uncorrected for a serum protein binding value of 15 ± 3% for ranitidine, calculated from plasma and urine ranitidine determinations (Carey, Martin & Owen (1980)) was 512 ml min^{-1}.

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The effect of acute renal failure on the pharmacokinetics of indocyanine green in the rat

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Renal failure in both man and experimental animals elicits changes in the hepatic biotransformation of drugs (Reidenberg, 1977; Terner, Wiebe, Noujaim, Dossetor & Sanders, 1978). However, it is possible that renal failure can affect other aspects of hepatic function, such as uptake and storage. We have, therefore, investigated the elimination of a non-metabolized dye, indocyanine green (ICG), in rats with acute renal failure (ARF).

ARF was induced in male Wistar rats (250–350 g) by withdrawal of drinking water for 24 h followed by an intramuscular injection of 10 ml/kg of 50% (v/v) glycerol in 0.9% saline. Control animals were similarly dehydrated and injected with 0.9% saline. Forty eight h after injection the rats were anaesthetized with

Table 1 Pharmacokinetic parameters of indocyanine green in control and uraemic rats

Parameter	*Controls* ($n = 6$)	*Uraemics* ($n = 6$)	% *Change*
$T_{\frac{1}{2}\alpha}$ (min)	1.6 ± 0.3	2.8 ± 0.4***	+75
$T_{\frac{1}{2}\beta}$ (min)	33.4 ± 4.8	51.5 ± 11.8**	+54
k_{12} (min^{-1})	0.430 ± 0.062	0.249 ± 0.041***	−42
k_{21} (min^{-1})	0.0066 ± 0.0014	0.0049 ± 0.001*	−26
k_{el} (min^{-1})	0.022 ± 0.003	0.015 ± 0.004**	−32
V_c (ml/100 g)	2.8 ± 0.3	3.3 ± 0.4*	+18
Plasma urea (mg/100 ml)	24 ± 3	98 ± 50**	+308

Mean ± s.d.; * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$, by two tailed Student's t-test.

pentobarbitone (60 mg/kg i.p.) and cannulae inserted into the trachea, right carotid artery and jugular vein. After the administration of ICG (7.5 mg/kg i.v.), serial blood samples (100 μl) were taken for up to 60 minutes. At the end of each experiment a further blood sample was taken for determination of plasma urea concentration. The concentration of ICG in plasma was determined by measuring its absorption at 800 nm.

The time course of the disappearance of ICG from plasma was found to be described by a two compartment model. Significant decreases in k_{12}, k_{21} and k_{el} were observed in uraemic rats (Table 1). There was a concomitant increase in plasma half times of the α- and β-phases and an increase in the volume of the central compartment (Vc). It is unlikely that these changes were due to decreased hepatic blood flow, because blood flow to the liver has been found to be increased in glycerol-induced ARF (Hiley, Yates, Roberts & Bloom, 1980). The decrease in k_{12} and k_{21} suggest that influx and efflux of ICG into and out of the hepatocyte is impaired. This may reflect a decrease in the permeability of the hepatocyte in renal failure.

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β_1 and β_2 Adrenoceptors in developing rat lung

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There is now substantial biochemical evidence for the co-existence of β_1 and β_2 adrenoceptors in lung tissue of several species (Barnett, Rugg & Nahorski, 1978; Rugg, Barnett & Nahorski, 1978; Minneman, Hegstrand & Molinoff, 1979). However, this receptor heterogeneity may merely reflect the heterogeneous cellular nature of the airways. In the present experiments using receptor labelling techniques, we have examined the density and relative proportion of β_1 and β_2 adrenoceptors in the developing rat in an attempt to associate the receptor subtypes with the development of different cell types in the lung. All experiments were performed on Wistar rats of either sex. Lungs were freed of major bronchi and membranes prepared essentially as previously described (Rugg, Barnett & Nahorski, 1978). The density of beta-adrenoceptors was determined using a rapid filtration technique and the radiolabelled antagonist [^{3}H]-(−)-dihydroalprenolol ([^{3}H]-DHA). Non-specific binding (binding remaining in the presence of 200 μM (−)-isoprenaline) was less than 20% at 0.5 nM[^{3}H]-DHA in all preparations. The dissociation constant (K_D) and receptor density (B_{max}) were determined by Scatchard plots.

The density of lung beta-adrenoceptors present at day 1 (99 ± 8 fmole/mg protein) increased gradually over the first 20 days (day 10, 177 ± 8 fmole/mg protein; day 20, 207 ± 6 fmole/mg protein). However, a more rapid development occurred over the next 10 days and a receptor density not significantly different from that in adult lung was observed at 30 days (470 ± 5 fmole/mg protein). The K_D for [^{3}H]-DHA (0.37 ± 0.09 nM in adult lung) was not significantly different at any age examined.

Estimation of the proportions of β_1 and β_2 adrenoceptors in lung membranes was made by computer-assisted analysis of the inhibition curves generated by the β_1 selective antagonists (±)-atenolol and (±)-betaxolol and the β_2 antagonist ICI 118.551. Despite the substantially different density of total beta-adrenoceptors at 2, 12 and 30 days postnatal, the proportions of β_1 and β_2 receptors were very similar at each age (day 2, 19% β_1 81% β_2; day 12, 26% β_1 74% β_2; day 30, 22% β_1 78% β_2; adult 22% β_1 78% β_2).

Since throughout the postnatal phase of lung development there is a marked cellular proliferation and differentiation (Thurlbeck, 1975) yet apparently a constant proportion of β_1 and β_2 adrenoceptors, both receptor subtypes may occur on the same cell type. Indeed, there is pharmacological evidence that both β_1 and β_2 adrenoceptors can mediate tracheal smooth muscle relaxation (Zaagsma, Oudhof, van der Heijden & Plantje, 1979).

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Inhibition of stimulation-evoked pressor responses in the pithed rat by dopamine agonists through activation of presynaptic dopamine receptors

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In the rat, as opposed to some other species, evidence for the existence of inhibitory presynaptic dopamine receptors, which modulate stimulation-evoked noradrenaline release from noradrenergic nerve endings, is limited to *in vitro* studies using vas deferens, tail artery and portal vein (Langer & Dubocovich, 1979). We have chosen the pithed rat to investigate further the possible existence of inhibitory presynaptic dopamine receptors on sympathetic nerve endings to the vasculature and heart of this species.

In pithed Sprague–Dawley rats the inhibitory actions of the dopamine agonists, 5,6 and 6,7 dihydroxy-2-dimethylamino tetralins (M-7 and TL-99 respectively) and N,N-dipropyldopamine (NNPD) on the frequency-dependent pressor responses and tachycardia evoked by electrical stimulation (supramaximal voltage for 20s at 0.25–8 Hz and pulse with 0.5 ms) of the entire sympathetic outflow from the spinal cord were determined; yohimbine (an α-adrenoceptor antagonist with some selectivity for α_2-adrenoceptors) and the dopamine antagonists, pimozide and metoclopramide, were used to investigate the nature of any inhibitory effects.

In the pithed rat M-7 and TL-99 (1, 3 and 10 μg/kg i.v.) caused pressor responses (TL-99 being about three times more active than M-7 as a stimulant of vascular post-synaptic α-adrenoceptors) and bradycardia whilst NNPD (1 mg/kg i.v.) caused a small pressor response and tachycardia: these responses were allowed to subside before commencing electrical stimulation of the spinal cord.

In groups of 6–9 pithed rats, M-7 (3 and 10, but not 1, μg/kg) inhibited by 50 to 90% the pressor responses and tachycardia evoked by low frequencies of stimulation whilst TL-99 (1–10 μg/kg) was inactive; NNPD (1, but not 0.3, mg/kg) only inhibited stimulation evoked pressor responses. Pimozide and metoclopramide (1 and 3 mg/kg i.v. respectively), but not yohimbine (1 mg/kg i.v.), prevented the inhibitory action of M-7 and NNPD on stimulation evoked pressor responses. Yohimbine (0.5 and 1 mg/kg i.v.), but not pimozide or metoclopramide, prevented the inhibitory action of M-7 on stimulation evoked tachycardia.

These findings provide evidence for the existence of inhibitory presynaptic dopamine receptors on nerve endings and/or ganglia of the sympathetic innervation to blood vessels in the rat; Cavero & Lefévre-Borg (1980) obtained similar results with NNPD in spontaneously hypertensive rats. As also showed by Hicks & Cannon (1979), inhibitory presynaptic α_2-adrenoceptors, and not dopamine receptors, modulate the effect of M-7 on stimulation evoked tachycardia in the rat. These results also demonstrate that N,N-dimethyl substitution of aminotetralins confers greater inhibitory activity at presynaptic receptors in the 5,6 rather than the 6,7 dihydroxy derivative whilst the converse applies for pressor activity.

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Antagonism of the inhibitory effects of clonidine and dopamine in the guinea-pig ileum by metoclopramide, sultopride and sulpiride

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The mechanism of the gastrointestinal stimulant effect of metoclopramide is unknown, although metoclopramide augments the responses to cholinergic postganglionic stimulation by a presynaptic mechanism (Hay, 1977) and blocks dopamine receptors (see Goldberg, Volkman & Kohli, 1978). The related benzamides sultopride and sulpiride are also dopamine receptor antagonists (Jenner & Marsden, 1979), but with less pronounced stimulant effects on the gastrointestinal tract (Fontaine & Reuse, 1978). As these drugs also interact with presynaptic α_2-adrenoceptors in the rat vas deferens (Spedding, 1980), their direct effects on field stimulation-induced concentrations of the guinea-pig ileum have been compared with their antagonist effects against drugs which specifically inhibit the responses to field stimulation by presynaptic mechanisms.

The contractile responses to field stimulation (0.05 Hz, 1 ms pulse duration, supramaximal voltage) of guinea-pig ileum preparations set up in Tyrode solution at 35°C were recorded isometrically. The Tyrode solution contained propranolol (0.5 μg/ml). Cumulative concentration-response curves to the agonists were obtained at 20 min intervals. Dose ratios were calculated from the ratio of the EC_{50} values for the agonist in the presence and absence of the antagonist.

The inhibitory effects of noradrenaline, dopamine and clonidine were antagonized to a similar extent by the antagonists (Table 1), whereas responses to 2-chloroadenosine were little affected. The parallelism between the antagonism of noradrenaline- and dopamine-induced inhibition by the three antagonists supports the proposal of Wikberg (1978) that dopamine acts on presynaptic α_2-adrenoceptors in this tissue. As metoclopramide was the only antagonist to augment the responses to field stimulation, this effect is unlikely to be related to antagonist effects at α_2-adrenoceptors or to antagonism of dopamine.

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Table 1 Augmentation of the contractile response to field stimulation (0.05 Hz) of guinea-pig ileum by benzamides and their antagonist effects on the inhibition of the response to field stimulation by clonidine, dopamine, noradrenaline and 2-chloroadenosine

Antagonist	*% Augmentation of contraction*	*Dose ratio for* *Clonidine*	*Noradrenaline*	*Dopamine*	*2-Chloroadenosine*
Metoclopramide					
10 μM	57 ± 4*	9.2 (7.9–10.8)*	5.1 (4.1–6.4)*	5.2 (3.2–8.4)*	—
100 μM	67 ± 7*	43 (17.8–105)*	18.3 (11.1–22.9)*	17.5 (11.9–25.5)*	1.6 (1.0–2.1)
Sultopride					
10 μM	4 ± 3	4.7 (4.3–5.5)*	6.2 (5.9–6.5)*	6.7 (4.3–10.8)*	—
100 μM	−1 ± 2	43.5 (11.2–83.8)*	13.5 (6.3–16.5)*	29.7 (20.5–43.4)*	0.8 (0.6–1.1)
Sulpiride					
10 μM	2 ± 3	3.8 (2.3–6.0)*	1.9 (1.1–3.4)	3.8 (3.4–4.3)*	—
100 μM	6 ± 3	34.5 (23.7–50.5)*	22.4 (14.3–35.6)*	32.1 (21.7–47.4)*	0.8 (0.6–1.1)

* $P < 0.05$, against control changes. Dose ratios are geometric means ± range s.e. mean, $n = 4$–6.

Clonidine-influence of aromatic substitution on α-adrenoceptor selectivity

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Starke, Endo & Taube (1975) observed that clonidine was a selective presynaptic α-adrenoceptor agonist. However, Medgett, McCulloch & Rand (1978) suggested that clonidine was a partial agonist on presynaptic and postsynaptic α-adrenoceptors. The present study describes the *in vitro* α-adrenoceptor selectivity of clonidine and some of its analogues. All studies were carried out in Krebs using tissues from male CFY rats (200–300 g).

In the selectivity studies presynaptic and postsynaptic α-adrenoceptor agonist potencies were determined on the vas deferens and anococcygeus muscle respectively (Doxey, 1979); the results are shown in Table 1. Two compounds, the 2,5 and 3,4 dichloro analogues potentiated the twitch response of the vas deferens (Table 1), with both compounds the potentiation was preceded by inhibition in approximately half of the experiments. The potentiation appeared to be the result of presynaptic antagonism since in the presence of prazosin (24 nM) the compounds antagonized the inhibitory effects of clonidine (3.7 nM) on the twitch response of the vas deferens. The concentrations of the 2,5 and 3,4 dichloro analogues and yohimbine which antagonised clonidine by 50% were 6.8 μM, 5.1 μM and 40 nM respectively. Both analogues of clonidine appear to be partial agonists at presynaptic α-adrenoceptors.

On the anococcygeus muscle and aorta, clonidine and these two analogues produced dose-related contractions but their maximum responses were significantly less than that produced by noradrenaline. These compounds appeared to be partial agonists at postsynaptic α-adrenoceptors since in the vas deferens and perfused mesentery they antagonized the effects of noradrenaline. All three compounds failed to induce vasoconstrictor responses in the mesentery.

In summary, aromatic substitution can influence α-adrenoceptor selectivity and in addition antagonist properties, particularly at the presynaptic site, become more apparent in individual compounds.

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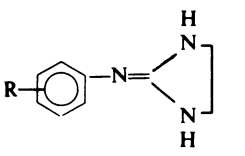
Table 1 Potencies of clonidine and some of its analogues at pre- and postsynaptic α-adrenoceptors. The results are the mean of a minimum of 3 experiments ± s.e. mean. Phentolamine (8.1 μM) was included in the Krebs at the end of each experiment to confirm that the agonist effects at both sites were mediated via α-adrenoceptors



R	*Presynaptic activity ED_{50}, inhibition of twitch response of rat vas deferens (M)*	*Postsynaptic activity ED_{50} concentration giving 50% maximum contraction anococcygeus muscle (M)*
—	$4.9 \pm 2.0 \times 10^{-5}$	$4.3 \pm 1.0 \times 10^{-6}$
2,6 dichloro (clonidine)	$2.2 \pm 0.6 \times 10^{-9}$	$2.3 \pm 0.9 \times 10^{-8}$
2,3 dichloro	$1.3 \pm 0.6 \times 10^{-9}$	$3.1 \pm 0.9 \times 10^{-7}$
2,6 diethyl	$4.5 \pm 0.9 \times 10^{-9}$	$2.7 \pm 1.6 \times 10^{-9}$
3,4 dihydroxy	$3.7 \pm 2.5 \times 10^{-9}$	$2.7 \pm 0.2 \times 10^{-8}$
2Me 5F	$6.0 \pm 3.0 \times 10^{-9}$	$1.6 \pm 0.6 \times 10^{-7}$
2,5 dichloro	Potentiation 3.7×10^{-7}	$5.0 \pm 3.0 \times 10^{-7}$
3,4 dichloro	Potentiation 1.1×10^{-6}	$1.0 \pm 0.4 \times 10^{-6}$

Preferential, long-lasting blockade of neuronally released but not exogenously administered noradrenaline *in vitro*; further evidence that the α_1-adrenoceptor subtype predominates intrasynaptically

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In the autoperfused hind-limb of the dog we have recently demonstrated the presence of α_2-adrenoceptors located postsynaptically (Langer, Massingham & Shepperson, 1980a). In addition, prazosin preferentially antagonized responses to neuronally released noradrenaline whilst it was much less effective in blocking responses to exogenously administered noradrenaline (Langer, Massingham & Shepperson, 1980b). In view of these findings we have suggested that the α_1-adrenoceptor in vascular smooth muscle of the dog may have a predominant location within the synapse (Langer, Massingham & Shepperson, 1980c).

Whilst we excluded an involvement of sympathetic, cholinergic vasodilator fibres, the perfused hind-limb preparation is a complex model and the preferential blockade by prazosin of responses to sympathetic nerve stimulation may be due to activation of other dilator mechanisms (e.g. histamine, dopamine). We have therefore sought an *in vitro* preparation in which to examine this action of prazosin and since we felt it important to study a complete vascular bed (ie blood vessels of varying caliber), we chose the isolated perfused cat spleen.

Cats (2–3 kg) were anaesthetized with pentobarbitone (60 mg/kg i.p.) and the spleen isolated, perfused with Krebs and set up for periarterial nerve stimulation as previously described (Dubocovich & Langer, 1980). The splenic sympathetic nerves were stimulated at supramaximal voltage using a 2ms pulse width at a frequency of 2 Hz; 120 shocks being delivered with each period of stimulation. After each stimulation, injections of noradrenaline (NA 2 μg) and phenylephrine (PHE 5 μg) were given to elicit approximately equal increases in spleen perfusion pressure (Nerve stimulation, 43 ± 13 mmHg, $n = 3$; NA, 55 ± 17 mmHg, $n = 3$; PHE 45 ± 8 mmHg, $n = 3$). Responses to nerve stimulation and exogenous amines were repeated 8 times at 20 min intervals and after several stable series of control responses, prazosin (3×10^{-8}M) was included in the Krebs for 20 min and the series of stimulations and injections repeated. The tissue was then perfused with prazosin-free Krebs and the recovery of the responses followed for 2 hours.

During exposure to prazosin responses to nerve stimulation were inhibited by $84 \pm 8\%$ and those to exogenous NA and PHE by $62 \pm 5\%$ and $90 \pm 2\%$ respectively. Furthermore after washout of prazosin responses to nerve stimulation, unlike those to exogenous NA, recovered only slowly. 20 min after prazosin washout responses to nerve stimulation were still inhibited $77 \pm 10\%$ whilst responses to injected NA were almost fully restored ($15 \pm 2\%$ inhibited, $P < 0.05$ Students t-test when compred to nerve stimulation). PHE responses at this time were $72 \pm 8\%$ antagonized, resembling the effects observed with nerve stimulation.

These results demonstrate that prazosin causes a long lasting inhibition of responses to sympathetic nerve stimulation in the cat spleen under conditions in which the overflow of [^{3}H]-NA elicited by nerve stimulation is not affected (See Massingham *et al.*, 1980). The preferential blockade of nerve induced responses could be explained if prazosin were taken up by sympathetic nerves and released on stimulation, thus producing a high concentration of drug in the vicinity of the receptors. This was investigated by pre loading spleens with [^{3}H]prazosin (Amersham, 28 Ci/mmol). Following a wash of 40 or 60 min in Krebs containing phentolamine (1 μM) the nerves were stimulated at 5 Hz for 1 min, and 20 min later NA (100 μg) was injected and this was followed by a further stimulation 20 min later. No release of tritium was detected to either stimulus, indicating that no release of [^{3}H]-prazosin took place. This is consistent with results obtained in the rabbit pulmonary artery (Cambridge, Davey & Massingham, 1977). Since the blockade of PHE responses follows a time course similar to that of nerve stimulation after prazosin the resuls suggest that NA released by nerve stimulation preferentially activates α_1-adrenoceptors. The faster recovery of responses to exogenous NA after prazosin-inhibition can be explained if exogenous NA, in contrast to neuronally released NA, stimulates postsynaptic α_1 and α_2-adrenoceptors. The occurrence of postsynaptic α_2- in addition to the classical α_1-adrenoceptor has recently been described in the rat (Timmermans, Kwa & Van Zwieten, 1980; Docherty & McGrath, 1980) and the dog (Langer *et al.*, 1980a, b, c).

In conclusion the finding that α_1-adrenoceptors predominate within the synapse in vascular smooth muscle neuroeffector junctions may explain the potency and effectiveness of prazosin as an antihypertensive agent. Furthermore the cat spleen appears to be a useful *in vitro* model in which to study this action of prazosin.

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The α- and β-adrenoceptor blocking potencies of labetalol and its individual stereoisomers

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Labetalol, an α- and β-adrenoceptor antagonist (Farmer, Kennedy, Levy & Marshall, 1972) contains two asymmetric centres and thus four stereoisomers can exist. The labetalol used for animal and human investigations is a mixture of equal proportions of the four stereoisomers. These stereoisomers have been stereospecifically synthesized (Clifton *et al.*, 1980) and this communication describes their α- and β-adrenoceptor blocking potencies.

The α_1-, β_1- and β_2-adrenoceptor blocking potencies of each stereoisomer was determined against phenylephrine-induced vasopressor responses and isoprenaline-induced positive chronotropic and vasodepressor responses in anaesthetized dogs (Brittain & Levy, 1976). Similar studies were carried out *in vitro* using

Table 1 Adrenoceptor-blocking potencies of labetalol and its stereoisomers enantiomers in anaesthetized dogs

Compound	*Vascular α_1-adrenoceptors*			*Cardiac β_1-adrenoceptors*			*Vascular β_2-adrenoceptors*		
	n	DR_{10}	*Slope*	*n*	DR_{10}	*Slope*	*n*	DR_{10}	*Slope*
Labetalol (RR, SS, SR, RS)	6	8.9 (6.2–12.7)	0.76 (0.69–0.83)	6	0.34 (0.27–0.43)	1.18 (1.09–1.28)	6	0.24 (0.13–0.45)	1.14 (0.87–1.5)
AH 19501 (RR)	5	58 (43–78)	1.03 (0.88–1.21)	7	0.15 (0.13–0.18)	1.10 (1.0–1.2)	7	0.11 (0.06–0.19)	1.23 (1.05–1.43)
AH 19502 (SS)	4	22.6 (13.4–38.1)	1.08 (0.79–1.49)	5	9.86 (5.46–17.8)	0.98 (0.64–1.49)	5	>10.0	—
AH 19503 (RS)	3	39.3 (19.7–78.5)	1.38 (0.96–1.99)	5	2.23 (1.19–4.19)	1.19 (1.01–1.41)	5	2.58 (1.76–3.79)	1.43 (1.11–1.85)
AH 19504 (SR)	5	5.11 (2.70–9.67)	0.75 (0.70–0.81)	5	7.95 (3.97–15.9)	1.01 (0.85–1.20)	5	11.31 (3.88–32.95)	0.95 (0.54–1.67)

Phenylephrine and isoprenaline were used as α- and β-adrenoceptor agonists respectively.
DR_{10} = intravenous dose of antagonist (mg/kg) required to produce an agonist dose-ratio of 10.
Slope = regression of the Arunlakshana and Schild (1959) plot.
DR_{10} and slope results are expressed as geometric mean (and 95% confidence limits)
n = number of determinations.

rabbit aortic strips (α_1-block) guinea-pig left atria (β_1-block) and guinea-pig tracheal strip (β_2-block).

The results from the *in vitro* and *in vivo* studies showed that the α- and β-adrenoceptor blocking properties of labetalol are not distributed equally amongst its stereoisomers. The RR stereoisomer (AH 19501) is a non-selective, competitive β-adrenoceptor antagonist about twice the activity of labetalol; this stereoisomer was virtually devoid of α-blocking activity. The SR stereoisomer (AH19504) exhibited the opposite profile of activity. The SS (AH19502) and RS (AH19503) stereoisomers have respectively weak α- and β-adrenoceptor blocking activity. The results obtained in anaesthetized dogs are summarised in Table 1. In concentrations approximately 1000 times greater than those required to block adrenoceptors, labetalol and its stereoisomers each caused concentration-dependent decreases in the relative refractory period of guinea-pig isolated left atria (Dawes, 1946). It is concluded that the therapeutic effects of labetalol in treating essential hypertension stem from a combination of the pharmacological activities of its stereoisomers.

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Apoyohimbine; a pattern for α-adrenoceptor antagonists or a template for the α-adrenoceptors?

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Rigid molecules in which the relationships of the component atoms are fixed due to the formation of fused five or six member rings can be of use in determining the structure/activity relationships required for a pharmacological effect. Yohimbine has such a structure, containing five fused rings in which rotation about the inter-atomic bonds can occur to a significant extent only in the cyclohexane ring E. In the pharmacologically active, naturally occurring forms of yohimbine there are several stereoisomers. Weitzell *et al.* (1979) have shown that as antagonists, several of these isomers have a marked 'preference' for the pre- or post- "synaptic" α-adrenoceptors in rabbit pulmonary artery, rauwolscine being relatively more effective at pre- (α_2) and corynanthine more effective at post-synaptic (α_1) receptors. In the rat vas deferens apoyohimbine, which is formed by hydrolysis of yohimbine at C 16–C 17, has been found to be more potent as an antagonist at either set of α-adrenoceptors than any other yohimbine isomer or derivative which has been tested (McGrath, 1980).

The structure of apoyohimbine is even more rigid than those of the yohimbine stereoisomers due to the introduction of a double bond into ring E. The object of the present study was to consider whether this structure could provide any clues to the structural requirements for blockade of the different types of α-adrenoceptor.

The potencies of α-adrenoceptor antagonists were assessed on rat, isolated vas deferens against the post-junctional contractile effect of neuronally released noradrenaline and the pre-junctional inhibitory effect of xylazine (McGrath, 1980). The stereochemistry of the antagonists was examined by building molecular models from information in the chemical literature.

Potency of antagonism at post-junctional α-adrenoceptors had the order: WB 4101 > prazosin > apoyohimbine > corynanthine > yohimbine > rauwolscine. At pre-junctional α-adrenoceptors the corresponding order was apoyohimbine > rauwolscine $\geqslant$ yohimbine > WB 4101 with the other two compounds having considerably less effect.

In molecular models of yohimbine stereoisomers or apoyohimbine reference points were taken as (1) the

aromatic ring A, (2) the tertiary N at position 4 (junction of rings C & D), (3) the C of the carboxyl group attached to C 16 in ring E: these three groups are precisely fixed in apoyohimbine by the molecular geometry. In yohimbine and rauwolscine these three points can be superimposed in the positions found in apoyohimbine without introducing undue steric hindrance or strained conformations. In corynanthine this relationship differs only in that (3) must remain slightly above the plane of ring E. In WB 4101 the structure can be arranged so that the aromatic part of the benzodioxane moiety corresponds to (1), the secondary amine link to (2) and an *o*-methoxy group on the phenyl ring to (3).

Based on the superimposition of apoyohimbine and WB 4101 conditions for antagonism at post- and prejunctional α-adrenoceptors are proposed in which two main areas of attachment are involved, viz. (a) (1)–(2), (b) the methoxy group at (3). From consideration of several antagonists, post-junctional (α_1) antagonism requires occupation at either (a) or (b) while pre-junctional (α_2) antagonism requires occupation at (a) but not (b) and can be prevented by the presence of bulky groups adjacent to (b).

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Contractile responses of the mouse anococcygeus muscle to some α-adrenoceptor agonists

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Motor responses of the mouse anococcygeus muscle to field stimulation and to exogenous noradrenaline (NA) are blocked by phentolamine, indicating that they are mediated by activation of postsynaptic α-adrenoceptors (Gibson & Wedmore, 1980). The present study examines in more detail the characteristics of the responses of the isolated tissue to four α-adrenoceptor agonists: NA, phenylephrine, clonidine and naphazoline.

Male mice (LACA; 25–35 g) were killed by stunning and exsanguination. Both anococcygeus muscles were set up in series, joined at the ventral bar, in organ baths (37°C) containing Krebs-bicarbonate solution (mM: NaCl 118.1, KCl 4.7, $MgSO_4$ 1.2; KH_2PO_4 1.2; $CaCl_2$ 2.5, $NaHCO_3$ 25.0, glucose 11.1). Contractile responses to the agonists were recorded isometrically, under a resting tension of 200 mg, the response being taken as the peak rise in tension produced by each dose. Antagonist dissociation constants were calculated by regression line analysis of Schild plots, the measurements being done in the presence of cocaine (2 μM) and corticosterone (10 μM). Some mice were pretreated with 6-hydroxydopamine (6-OHDA. 2 × 50 mg/kg on day 1 i.p.; 2 × 100 mg/kg on day 4 i.p.; experiment on day 5). The effectiveness of this treatment was confirmed by fluorescence microscopy and by lack of motor responses to field stimulation and to tyramine.

All four agonists produced dose-related contractions of the mouse anococcygeus muscle. The dose-response curves to NA and phenylephrine showed a 10-fold shift to the left following 6-OHDA pretreatment or in Krebs solution containing a mixture of cocaine (2 μM) and corticosterone (10 μM). Neither of these procedures altered the dose-response curves to clonidine or naphazoline. In the presence of the uptake blockers the $-\log_{10}ED_{50}$ (M) and maximum response (mg) of the agonists were (mean ± s.e. mean°: naphazoline 7.27 ± 0.05; 683 ± 52; clonidine 7.24 ± 0.05, 676 ± 45; NA 6.48 ± 0.07, 622 ± 34; phenylephrine 6.47 ± 0.08, 599 ± 47. In addition, the responses to naphazoline and clonidine were more prolonged, the mean time to peak response being about 5–6 min compared to 1–2 min for NA and phenylephrine. The antagonist potencies of prazosin and of yohimbine were studied, and the dissociation constants measured against naphazoline, clonidine, NA, and phenylephrine respectively were: for prazosin, 16 nM, 16 nM, 10 nM, and 20 nM; for yohimbine, 2.2 μM, 1.0 μM, 1.6 μM, and 2.0 μM.

Thus, in the isolated anococcygeus muscle of the mouse clonidine and naphazoline are more potent direct postsynaptic α-adrenoceptor agonists than are

NA and phenylephrine, and produce contractions of much longer duration. The receptors mediating the responses to all four agonists are about 100 times more susceptible to block by prazosin than by yohimbine. The cellular mechanisms underlying postsynaptic α-adrenoceptor stimulation in this tissue are being investigated.

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Time-course of the cardiac effects of a single dose of L-3,3',5-triiodothyronine in thyroidectomized rats

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Administration of thyroid hormones to animals induces many changes in function including an increase in metabolic rate, increased synthesis of enzymes and stimulation of the heart. There is a characteristic latent period before any of these effects can be observed. For example, basal metabolic rate does not increase until 20 to 30 h and enzyme activity, such as glucose-6-phosphatase, only increases after 12 to 36 h (Tata, Ernster, Lindberg, Arrhenius, Pedersen & Hedman, 1963). The following experiments were designed to assess the time course of changes in cardiac function following a single injection of L-3,3'5-triiodothyronine (L-T_3).

Thyroidectomized (Tx) rats were given a single subcutaneous injection of L-T_3 (500 μg/kg) in alkaline saline or vehicle alone. At 12, 18, 24, 48 or 96 h, rats were killed and the hearts perfused using a modification of the isolated working heart apparatus described by Neely, Liebermeister, Battersby & Morgan (1967). Hearts were perfused via the left atrium, at a filling pressure of 10 cm H_2O, with modified Krebs-Henseleit bicarbonate buffer containing Ca^{2+} (1.25 mM) at 33°C. The left ventricle ejected fluid against a pressure head of 70 cm H_2O. Contractility was assessed using the maximum value of the first differential of left ventricular pressure (dLVP/dt max). Spontaneous heart

Table 1 Cardiac function at different times after injection of L-T_3 (500 μg/kg). Values shown are mean ± s.e. mean. Statistical significance compared with vehicle injected controls was tested using Student's '*t*'-test and the levels of significance shown

Time after injection	*SHR (beats/min)*	*dLVP/dt max (mmHg/s at 300 bpm)*	*Cardiac output (ml min^{-1} g dry weight of heart at 300 bpm)*
12 h ($n = 6$)	178 ± 7	4330 ± 330[b]	225 ± 12
18 h ($n = 6$)	199 ± 11	4390 ± 180[a]	278 ± 6[b]
24 h ($n = 5$)	220 ± 3[a]	4430 ± 230	254 ± 14
48 h ($n = 6$)	256 ± 10[a]	5590 ± 330[a]	266 ± 13
96 h ($n = 5$)	250 ± 9[a]	4420 ± 350[b]	296 ± 30
Vehicle alone ($n = 6$)	170 ± 5	3210 ± 150	226 ± 16

a—$P < 0.001$, b—$P < 0.025$, compared with controls.

rate (SHR) was recorded before measurements of cardiac function were made in hearts paced electrically at 300 beats/min. Increasing the heart rate from spontaneous to 300 beats/min did not significantly affect dLVP/dt max. Results are summarized in Table 1. At 12 h, only dLVP/dt max was significantly increased. Other parameters measured did not increase until 18 hours. All parameters were maintained at or near maximal values at 48 hours.

The results indicate that the rise in contractility occurs earlier than reported for other effects of thyroid hormones and that there is a difference between the time course of the increase in SHR and the increase in contractility.

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A comparative study of the effect of sulphonylureas on regional blood flow determined by a radioactive microsphere technique in experimentally-induced diabetes in the rat

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Alterations in blood flow may be a contributory factor in the pathogenesis of diabetic vascular disease. Regional blood flow changes have been demonstrated in the experimentally diabetic rat (Foy & Lucas, 1977). The aim of this study was to determine whether treatment with oral sulphonylureas modified these blood flow changes.

Male rats (approx. 200 g) were rendered diabetic by rapid tail vein injection of either alloxan (50 mg/kg) of streptozotocin (60 mg/kg). Control animals received vehicle only. Gliclazide (50 mg/kg/day) and glibenclamide (5 mg/kg/day) were administered via the diet (0.1% and 0.01% w/w respectively) for two days in the case of alloxanised rats or 12 days for those given streptozotocin. Blood flow was determined under pentobarbitone anaesthesia (40–60 mg/kg I.P.) using a radioactive microsphere technique. Sc-46 labelled microspheres of 15 microns diameter and known radioactivity were injected via a carotid cannula directly into the left ventricle of the heart. Cardiac output and regional blood flow was determined from the activities of blood withdrawn and from organ samples. Although blood flow to many regions was not significantly modified by drug treatment, that to the eyes and kidney of streptozotocin diabetic rats treated with gliclazide was significantly elevated (see Table 1). Further studies are progressing in order to elucidate these findings.

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Table 1 Blood flow (ml/min/100 g) to the eye and kidney of the rat (mean ± s.e. mean). Pairs of characters indicate significant differences (at least $P > 0.05$) between the two values

	Kidney	*Eye*
Control	428 ± 20*	83 ± 14*†
Alloxan	256 ± 24*†	48 ± 3†
Streptozotocin	392 ± 23‡	52 ± 4*†‡
Alloxan + gliclazide	275 ± 30	49 ± 9
Alloxan + glibenclamide	330 ± 30†	58 ± 8
Streptozotocin + gliclazide	496 ± 30‡	85 ± 9‡
Streptozotocin + glibenclamide	376 ± 30	53 ± 6